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THE NATURAL IMMUNITY OF THE LARCH SAWFLY (*PRISTIPHORA ERICHSONII* (HTG.)) TO THE INTRODUCED PARASITE *MESOLEIUS TENTHREDINIS* MORLEY, IN MANITOBA AND SASKATCHEWAN[1]

BY J. A. MULDREW[2]

Abstract

By 1945 it was suspected that the larch sawfly in Saskatchewan and Manitoba had developed an immunity to the introduced parasite *Mesoleius tenthredinis* Morley. Results of subsequent studies showed that embryonic development of *M. tenthredinis* in sawfly larvae from Manitoba and Saskatchewan was inhibited three to four days after oviposition. No such inhibition occurred in larvae from British Columbia where the parasite is still highly effective. Inhibition seemed to be related to the deposition of phagocytic capsules around parasite embryos, which occurred in host larvae from Manitoba and Saskatchewan but not in larvae from British Columbia. Encapsulated parasite embryos were shown to be viable, for a number of them hatched after being placed in Ringer's solution. Viability decreased with age. Some embryos were viable seven months after oviposition, though the normal incubation period is 7 to 10 days. Unencapsulated embryos developed more rapidly than encapsulated embryos in Ringer's solution. Results obtained indicate that the phagocytes of the larch sawfly from Manitoba and Saskatchewan play an important role in the immunity reaction of this insect to *M. tenthredinis*.

Introduction

The larch sawfly is the most important pest of tamarack (*Larix laricina* (Du Roi) (K. Koch)) in North America. Available evidence indicates that this insect was introduced from Europe and that it has probably been present in North America since about the beginning of the nineteenth century (4, 11). Since 1882 a number of devastating outbreaks of the insect have occurred in North America. The current outbreak began in Manitoba about 1938 and now covers extensive areas in Saskatchewan, Manitoba, Ontario, and the Lake States.

The parasite *Mesoleius tenthredinis* Morley was first imported into Canada from England in 1910 and 1911 and liberated in Ontario and Quebec (11). The liberations made in Quebec were reported to be highly successful (10). Parasites were released in the Riding Mountain National Park, Manitoba, in 1912, and in the Spruce Woods Forest Reserve, Manitoba, in 1913. Examinations of cocoons in the latter area in subsequent years showed the following

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Contribution No. 89, Division of Forest Biology, Science Service, Department of Agriculture, Ottawa, Canada. Based on a thesis presented to the Faculty of Graduate Studies and Research of the University of Manitoba, in April, 1952, in partial fulfillment of the requirements for the degree of Master of Science.

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[The June number of this Journal (Can. J. Zool. 31: 167-312. 1953.) was issued July 8, 1953.]

increases in percentage of effective parasitism by *M. tenthredinis:* 1914—none recovered; 1916—19%; 1917—22%; 1919—40%; 1920—66% (4). The larch sawfly then became scarce in this area and no further studies were made until 1926 when a series of sawfly cocoons were gathered about 10 miles from the locality where the parasites had originally been liberated. Sixty-six per cent of these were parasitized. In 1927 parasitism reached 88% in some parts of the Spruce Woods Forest Reserve and averaged 75% over the whole area.

It became apparent in Manitoba about 1940 that the effectiveness of *M. tenthredinis* had decreased greatly. The superficial cause was the failure of most of the *M. tenthredinis* eggs deposited in host larvae to hatch. Although a high percentage of sawfly larvae were frequently parasitized by *M. tenthredinis*, the number of parasites reaching maturity and killing their hosts was very small, rarely exceeding 5%.

A record in 1945 (1) stated that all the unhatched eggs were completely surrounded by "thick translucent coatings, presumably laid down by the action of the host". It was felt that this phenomenon might have a bearing on the decreased effectiveness of *M. tenthredinis*. From 1949 to 1951 the author conducted an investigation on this aspect of the problem. This paper describes the results of this study. Research was conducted in the Riding Mountain National Park, the Whiteshell Forest Reserve, and Winnipeg, Manitoba, and at Prince Albert, Saskatchewan.

Life Cycle of *Mesoleius tenthredinis*

There is only one generation of *M. tenthredinis* per year. The parasites overwinter within the cocooned host larvae. The majority of the hibernating parasite larvae appear to be third- and fourth-instars. In the spring the parasites resume feeding, and when mature, they pupate within the larch sawfly cocoons. In Manitoba and Saskatchewan the adult parasites emerge from late June to early August and after mating, the females search out the sawfly larvae and deposit eggs internally, mainly into third-, fourth-, and fifth-instar larvae of the host. The eggs of *M. tenthredinis* normally hatch in 7 to 10 days and the young larvae then feed, grow, and molt, until they hibernate.

History of the Immunity Reaction Observed in the Larch Sawfly

From 1938 to 1940, 11,462 larch sawfly cocoons were collected in the Spruce Woods Forest Reserve. The total parasitism determined by emergence from incubated cocoons was only 0.017% and parasitism was caused entirely by the tachinid *Bessa harveyi* Tns. (24). *M. tenthredinis* was seemingly absent from this area in which it had been so prevalent 10 years previously.

Overwintering larch sawfly larvae were dissected in 1941 and it was found that most of the larvae that harbored *M. tenthredinis* contained unhatched eggs of the parasite but no living larvae of the parasite. The results of these dissections are given in Table I, where "total parasitism" refers to host larvae containing parasite eggs or larvae, and "effective parasitism" refers to host larvae containing parasite larvae.

TABLE I

PARASITISM OF THE LARCH SAWFLY BY *M. tenthredinis* IN 1941 AND 1942 IN MANITOBA

Year	S.W.F.R.*		R.M.N.P.**		S.W.F.R.			R.M.N.P.		
	No. of cocoons incubated	Parasitism, %	No. of cocoons incubated	Parasitism, %	No. of larvae dissected	Total parasitism, %	Effective parasitism, %	No. of larvae dissected	Total parasitism, %	Effective parasitism, %
1941	1111	0.1	23,003	5.1	200	2.5	2.0	400	30.0	7.5
1942	—	—	10,000	4.3	—	—	—	—	—	—

* *Spruce Woods Forest Reserve, Manitoba.*
** *Riding Mountain National Park, Manitoba.*

Data collected since 1945 by the Forest Insect Laboratory at Winnipeg, from dissections of cocooned larch sawfly larvae, indicate that the effective parasitism has remained low. This trend is illustrated in Fig. 1, which shows the total and effective parasitism by *M. tenthredinis* in three representative areas in Manitoba and Saskatchewan.

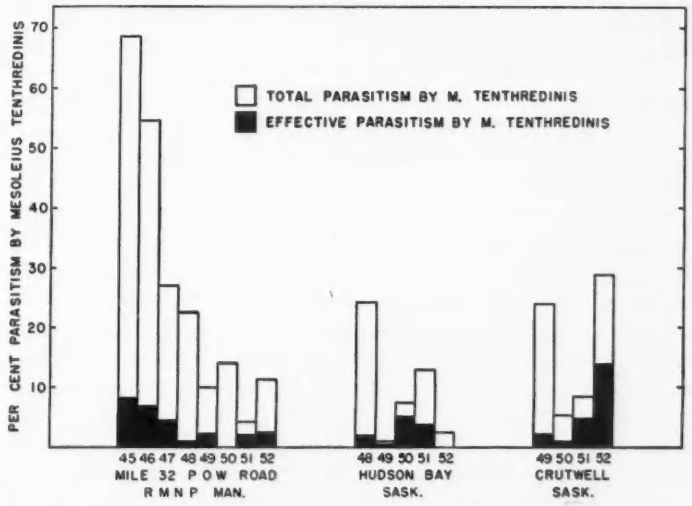

FIG. 1. Trends in total and effective parasitism by *M. tenthredinis* in two areas in Saskatchewan and one in Manitoba.

Review of the Literature on the Immunity of Insects to Insect Parasites

Bess (2) discussed the use of the word "phagocytosis" in connection with the encapsulation of insect parasite eggs and larvae. He believed that whereas the formation of agglomerations of cells around eggs and larvae of internal

parasites is an extracellular phenomenon and the ingestion of small particles by phagocytes is an intracellular phenomenon, nevertheless the reactions are probably the same in principle.

Bess pointed out that there are two main viewpoints about the nature of this type of immunity:

(1) that the phagocytes attack and encapsulate living parasite eggs and larvae and are a primary cause of their death; and

(2) that immunity is based on some humoral phenomenon in which the phagocytes act merely as scavengers, protecting the host against injury from unhealthy and dead parasite eggs and larvae.

Cuénot (5) supported the first view, as did Timberlake (31), who believed that unadapted parasites cannot repel phagocytes and may succumb to them regularly. A successful parasite, he suggested, is either so similar to the host in physical being that its presence is not resented, or secretes substances into the blood that paralyze the protective reactions of the host.

Chorine (3) found that pathogenic protozoa will develop successfully only in their proper insect hosts, where there is little phagocytosis. Ephrussi and Beadle (7) and Wigglesworth (34) found that implants of living tissue from the same or allied species can live and develop without molestation by phagocytes. Ries (23) showed that implants from unrelated species may be the center of considerable hematocytic activity. This evidence supports the view that the protoplasm of unsuccessful parasites is incompatible with that of the host.

Strickland (25, 26) found that embryos of *Meteorus vulgaris* Cress. were overcome by phagocytes only when introduced into pale western and red-backed cutworms just prior to pupation, and postulated that they could not gain sufficient immunity to resist the increased phagocytic activity at this time. His work on leaf-ovipositing tachinids led him to postulate that the larvae of all such parasites gain immunity to the phagocytic action of the host by burying themselves in some structure such as a ganglion or muscle fiber immediately after entering the body cavity of the host.

Mellanby (16) was of the opinion that the attraction of the phagocytes to an invading body is chemotactic, dependent upon the liberation of attractive substances. He considered that the absence of phagocytosis indicates that a parasite is adapted to its host. Wigglesworth (32) commented on mammalian blood cells remaining unchanged for weeks in the hemocoele of the bed bug and Mellanby suggested that these cells do not emit chemotactic substances.

Pemberton and Willard (21) found that the melon fly normally encapsulated and killed the eggs of a parasite "A" except when prior oviposition by a parasite "B" occurred. They concluded that the immunization of the melon fly to "A" was destroyed by the injection of toxic substances of the adult female of "B" at oviposition. Bess (2) cited evidence to contradict this theory. He also obtained evidence indicating that in some instances phagocytosis is initiated by substances liberated by the parasite about the time of hatching. However, encapsulation does not always occur at the time of hatching (18, 30).

Paillot (19) concluded that the phagocytic reaction in corn-borer larvae definitely caused the death of certain parasite eggs.

Pantel (20) and Thompson (27, 28, 29) supported the second main viewpoint mentioned at the beginning of this review, namely, that healthy parasites do not have to secrete antiphagocytic substances in order to survive, since the phagocytes of arthropods are not inherently disposed to attack invaders but react only to pathological exudates resulting from the degeneration of cells. Mellanby (16) suggested that probably the main normal function of the phagocytes is the removal of dead tissues. This idea is supported by Wigglesworth's work on *Rhodnius* (33). There is some controversy (8, 16, 22) as to whether the phagocytes actually initiate the breakdown of tissues during pupation or whether they are attracted by the first chemical products of the breakdown. Bess (2) found that immunity is not necessarily accompanied by phagocytosis and concluded that phagocytosis is not a primary factor in immunity but independent of it.

In addition to the two main viewpoints discussed, other hypotheses have been advanced. Several workers (2, 9, 16, 18) have postulated that the immunity of a host to a parasite may be the result of an unsuitable food medium in the host which causes the parasite to succumb to malnutrition, or starvation. Hollande (12) considered a combination of factors involving nutrition, secretion of phagocyte-repelling substances by the parasite, and secretion of phagocyte-attracting substances by the capsule.

A number of workers (2, 14, 17, 31) have found healthy parasite eggs and larvae completely encapsulated. Sometimes the embryos complete development and the small larvae escape from the capsule (14), though the incubation period of such eggs may be abnormally long (2, 14). More often the living encapsulated parasites eventually die (2). Thorpe (30) obtained evidence which indicated that phagocytic capsules may destroy enclosed parasites by stopping or reducing the flow of gases to and from these parasites.

"Melanization" has been considered by various workers (2, 6, 30) as a defense reaction of insects to internal parasites.

Normal health and longevity are apparently experienced by adult hosts containing encapsulated parasites (21, 31).

Studies on the Natural Immunity of the Larch Sawfly to *M. tenthredinis*

Host Stage Attacked and Seasonal Period of Attack in Relation to Effective Parasitism by *M. tenthredinis*

In 1945 two explanations for the poor hatch of *M. tenthredinis* eggs were advanced: (*a*) that possibly the parasite eggs were laid in late-stage sawfly larvae; and (*b*) that they did not have time to hatch before the larvae had spun their cocoons. Another possibility suggested was that the eggs were laid late in the season, and did not have time to hatch before cold weather began (1).

To test these hypotheses, work was carried out in the Riding Mountain National Park, Man., in 1949, and near Prince Albert, Sask., in 1950. Larch sawfly larvae of each instar present in the field were collected at weekly intervals and dissected. Some larvae were dissected in the field; others were reared to the cocoon stage and dissected during the winter. The data obtained were combined and are presented in Fig. 2.

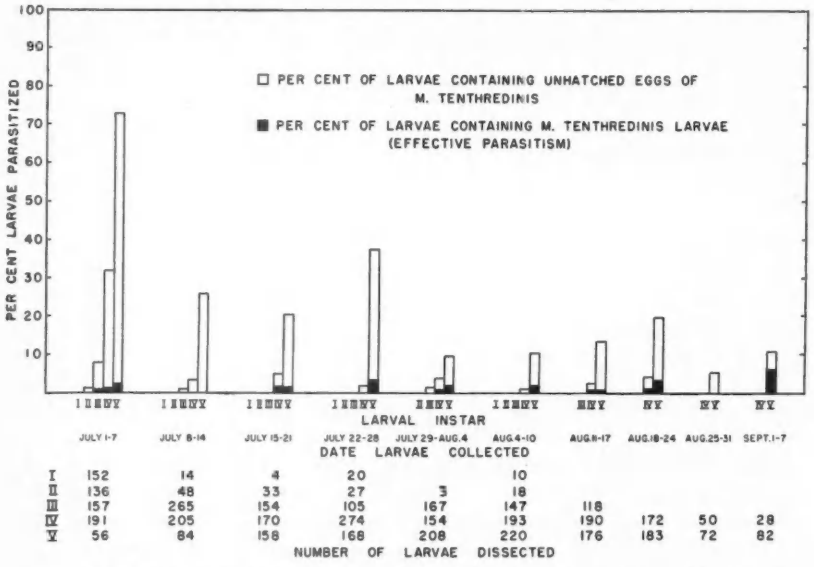

FIG. 2. Seasonal trend of the total and effective parasitism of the larch sawfly by *M. tenthredinis*. Where no column appears above an instar number, none of the larvae dissected were parasitized.

There is no obvious relation between the larval stage attacked and the effective parasitism by *M. tenthredinis* since parasite larvae were found in third-, fourth-, and fifth-instar larvae of the larch sawfly. There is also no obvious relation between seasonal period of attack and the effective parasitism by *M. tenthredinis*, since parasite larvae were found in host larvae throughout the season from the first week in July to the first week in September. First-instar larch sawfly larvae were not attacked by *M. tenthredinis* and second- and third-instar sawfly larvae were attacked only to a very slight degree.

IMMUNITY STUDIES ON HOST MATERIAL FROM BRITISH COLUMBIA

History of M. tenthredinis in British Columbia

The larch sawfly was first noted in British Columbia in 1930. In 1933, cocoons were collected and the larvae dissected. No evidence of parasitism by *M. tenthredinis* was found (13). During 1934, 1935, and 1936, 3650 *M. tenthredinis* adults were released in British Columbia. The parasite became

established and spread with the sawfly. In 1948 and 1949, dissections revealed that 60–70% of the sawfly larvae in British Columbia were parasitized effectively by *M. tenthredinis* (15).

Immunity of Host Material from British Columbia

During the winter of 1950-1951 a collection of 250 larch sawfly cocoons from British Columbia was supplied for experimental purposes through the kindness of J. H. McLeod, Insect Parasite Laboratory, Vancouver, B.C. Adult sawflies were obtained from these cocoons and the larval progeny from these adults were reared in the Whiteshell Forest Reserve, Man., for controlled parasitism experiments. Groups of these larvae were parasitized by *M. tenthredinis* obtained from Saskatchewan, and *M. tenthredinis* from British Columbia. Concurrently, larch sawfly larvae collected in the Whiteshell Forest Reserve were also parasitized by these same parasites. These larvae were dissected 10 or more days after parasitization. The results are presented in Table II.

TABLE II

DISSECTION RESULTS OF LARCH SAWFLY FROM DIFFERENT SOURCES PARASITIZED BY *M. tenthredinis* FROM DIFFERENT SOURCES

Source of sawfly larvae parasitized	Instar parasitized	Source of *M. tenthredinis*	No. of parasitized sawfly larvae dissected	Per cent of hosts containing a living larva of *M. tenthredinis* (effective parasitism)	Hosts containing only unhatched eggs of *M. tenthredinis*	
					With capsules	Without capsules
B.C.	IV	B.C.	7	100	0	0
B.C.	V	B.C.	5	100	0	0
B.C.	IV	Sask.	7	100	0	0
B.C.	V	Sask.	34	94	2	0
Man.	V	B.C.	15	6.6	14	0
Man.	V	Sask.	13	7.7	12	0

In the 53 British Columbia larch sawfly larvae parasitized by *M. tenthredinis*, the parasite developed normally in 51 (or 96%) and in these hosts no encapsulation occurred. In 2 (or 4%) of these 53 larvae, development of the parasite was arrested and these hosts contained encapsulated eggs. In contrast to this situation *M. tenthredinis* develops normally in relatively few hosts in Manitoba and Saskatchewan where a high degree of encapsulation of *M. tenthredinis* eggs occurs.

In 38 parasitized sawfly larvae from British Columbia that were removed from cocoons and dissected during the winter, 37 living *M. tenthredinis* larvae were found in 36 of the hosts. In one host two living parasite larvae were found, one of which was considerably larger than the other. Besides the 37 living larvae, 23 dead, unencapsulated, first-instar larvae and 11 dead, unencapsulated eggs were found. It appeared that the first parasite larva

to emerge was, in some way, able to kill the rest of the *M. tenthredinis* that occurred in the same host. Evidently, in most cases the supernumerary parasites were killed as first-instar larvae shortly after they emerged from the chorion. In some instances, dead first-instar larvae were found with the anterior end of the larva projecting out of the chorion. A number of *M. tenthredinis* embryos were killed while still within the chorion. In no case were any of the supernumerary *M. tenthredinis* found encapsulated within larch sawfly larvae from British Columbia. In a few instances "foreign bodies" were found in sawfly larvae from British Columbia and these were encapsulated.

Viability of Encapsulated Embryos of *M. tenthredinis*

This study was carried out at Prince Albert, Sask., during the summer of 1950. Prior to 1950 the author had observed that many of the encapsulated parasite eggs removed from sawfly larvae 10 days or more after parasitization contained apparently healthy, partly developed embryos. Many of these appeared to be at approximately the same stage of development.

During July, 1950, an attempt was made to determine whether these embryos were still viable. Viability could be demonstrated if the encapsulated embryos could be made to resume development when suitable conditions were provided. It was postulated that the phagocytic capsules prevented the embryos from completing their development within the sawfly hosts. Ten encapsulated eggs were removed from parasitized sawfly larvae and the capsules were carefully removed. The capsule-free eggs were then placed in Ringer's physiological solution.* They were examined periodically and further embryonic development was observed in all but one. Approximately five days after the eggs had been removed from their hosts and placed in the Ringer's solution nine had hatched. The small first-instar larvae died about two days after eclosion, presumably from starvation. Further small-scale studies revealed that the embryos would resume development and hatch in Ringer's solution even when the capsules were not removed. More extensive studies were then carried out as described below.

Effect of Time Spent in Host on Viability of M. tenthredinis Eggs

In the summer of 1950, larch sawfly larvae collected near Prince Albert, Saskatchewan, were parasitized in cages by adult *M. tenthredinis* and were dissected at intervals following parasitization. The parasite eggs found were placed in small glass dishes each containing about $\frac{1}{10}$ cc. of Ringer's solution. These were covered by inverted glass jars to prevent excessive evaporation, and the whole covered by a metal container to shade the eggs from bright light.

* *Formula: NaCl, 8.0 gm.; $CaCl_2$, 0.2 gm.; KCl, 0.2 gm.; $NaHCO_3$, 0.2 gm.; distilled water 1000 ml. The embryos also hatched in other physiological solutions but insufficient work was done to determine the optimum solution. Ringer's and all of the other solutions tested appeared to be hypotonic*

A summary of the data obtained is presented in Table III. The various lots of *M. tenthredinis* eggs treated during the experiment are grouped into 10 units based on the time spent by the parasite eggs in the sawfly larvae.

TABLE III

THE RELATIONSHIP BETWEEN THE TIME SPENT BY *M. tenthredinis* EGGS IN SAWFLY LARVAE AND THE VIABILITY OF EGGS

Time (in days) spent by parasite eggs in sawfly lavae	Total no. of eggs	Total no. of parasite larvae found at dissection	Hatch of parasite eggs within the sawfly larvae, %	Eggs having a visible capsule, %	Hatch of eggs in Ringer's solution, %
0 to 3	338	0	0.0	3.5	82.5
3 to 5	102	0	0.0	43.0	85.3
5 to 10	373	8	2.1	73.7	76.1
10 to 15	336	44	11.6	72.9	67.9
15 to 20	96	3	3.0	90.6	68.7
20 to 30	119	3	2.5	100	37.0
50 to 60	80	1	1.2	100	20.0
75 to 80	38	0	0.0	100	26.3
156	40	1	2.4	100	10.0
217	35	0	0.0	100	2.9

Table III indicates that the factor or factors responsible for preventing the *M. tenthredinis* eggs from developing and hatching in the sawfly hosts does not kill many parasite eggs immediately but only inhibits growth and differentiation of the parasite embryo. This evidence would seem to favor the view that phagocytes can attack healthy parasites as expounded by Cuénot (5) and Timberlake (31). The data further reveal that as the length of time *M. tenthredinis* eggs spend in the host larvae increases, the proportion of the eggs found to be encapsulated increases and the viability of the eggs decreases.

That encapsulated *M. tenthredinis* eggs removed from larch sawfly larvae hatch in Ringer's solution even when the capsules are left on these eggs does not necessarily indicate that the capsules are not the main factor responsible for inhibiting the development of the parasite embryos. The cells composing the capsule may have been alive when in the host larvae but may have died after transfer to the Ringer's solution and it may be necessary for these cells to remain alive in order to inhibit effectively the growth of the *M. tenthredinis* eggs.

After the encapsulated eggs were transferred from the sawfly larvae to the Ringer's solution, it was noted that the chorions of many of these eggs expanded considerably, probably because the Ringer's solution was hypotonic, and often split the capsule open at one end and protruded from the opening. The parasite embryos were often found adjacent to that part of the chorion that protruded from the capsule. These observations may indicate that the phagocytic capsules exert a detrimental effect on the embryos due to a permeability

phenomenon affecting the availability of oxygen and/or the elimination of toxic materials. Some embryos, however, developed and hatched in capsules that did not appear to be split.

Most of the encapsulated eggs that were removed two or more months after oviposition were found to be dead. However, none of the groups of parasite eggs placed in Ringer's during this study showed complete mortality. One egg hatched from a group of 35 removed seven months after oviposition. Evidence obtained from other studies indicates that none of the encapsulated eggs removed eight or more months after parasitization were viable.

The normal incubation period of *M. tenthredinis* is about 7 to 10 days but parasite embryos can survive in host larvae for seven mouths. A quiescent period of such length resembles diapause but here the embryos develop normally shortly after being placed in Ringer's solution. This seems to indicate that the quiescence is a response to unfavorable conditions and not true diapause.

The percentage of parasite eggs found to be encapsulated at dissection increased as the time spent by these eggs in the host larvae increased. Table III indicates that 100% encapsulation was not reached until after the eggs had spent 20 days in the sawfly hosts. This table is based on dissections of both healthy and unhealthy host larvae. A lower percentage of eggs were encapsulated in unhealthy host larvae than in healthy host larvae. In healthy host larvae approximately 100% of the eggs were encapsulated after a 10-day period. The reactions of unhealthy host larvae are discussed in more detail in a later section of this paper.

Unencapsulated eggs were found in apparently healthy host larvae, in which no living *M. tenthredinis* larvae were present, 15.5 days after parasitization. Transfer of these eggs to Ringer's solution showed that they were not all dead, as some developed and hatched. Since the normal incubation period of *M. tenthredinis* eggs is approximately 7 to 10 days it is difficult to explain why these unencapsulated eggs had not hatched 15 days after parasitization. It was the presence of unencapsulated, unhatched, parasite eggs discovered by Bess (2) during his studies on the immunity of mealy bugs to insect parasites that caused him to conclude that "phagocytosis is not a primary factor in immunity but independent of it". The presence of these unencapsulated, unhatched, parasite eggs in larch sawfly larvae appears to discredit the theory that the phagocytic capsules inhibit the development of *M. tenthredinis* embryos and seems to add support to the hypothesis that a chemical substance produced by the host inhibits the development of *M. tenthredinis* embryos in larch sawfly larvae.

It may be, however, that capsules had formed about these unencapsulated eggs, inhibited development, and then the cells forming the capsule may have dispersed before the larvae were dissected. These eggs hatched very shortly after being placed in the Ringer's solution as is shown in Fig. 3.

The hemocytes that float free in the blood stream of insect larvae are known to increase greatly during molting. One of the possibilities contemplated was

the occurrence of a significant increase in the rate of deposition of the capsule during ecdysis. The evidence obtained from this study was not conclusive, but it indicated that, proportionately speaking, eggs were encapsulated more readily in fourth-instar larvae than in third-instar larvae and more rapidly in third-instar larvae than in fifth-instar larvae. This seems to relate to the results shown in Fig. 2 of this paper which shows no obvious relation between the sawfly instar attacked and the effective parasitism by *M. tenthredinis*.

Occasionally encapsulated *M. tenthredinis* embryos develop and hatch within host larvae. In one host larva, dissected 29 days after parasitization, two living first-instar parasite larvae were found; beside them were two empty encapsulated chorions. However, in nearly all instances where first-instar larvae had hatched within host larvae, the empty chorions were found to be unencapsulated. In no cases were first-instar larvae found to be encapsulated and this was true for both living and dead first-instar parasite larvae.

Some evidence was found which indicated that the small first-instar parasite larvae were less resistant to phagocytic digestion than were the parasite embryos within the chorions. In four cases empty unencapsulated chorions were found from which parasite larvae had apparently emerged. The parasite larvae themselves, however, were not found within these host larvae, despite a painstaking search of the internal contents of each host. The small parasite larvae may have been completely devoured by phagocytes. No empty phagocytic cysts or empty first-instar larval integuments were found in these hosts.

Effect of Presence or Absence of Capsules on Hatching Time of M. tenthredinis Eggs

In a number of trials during the experiment discussed above, *M. tenthredinis* eggs removed from groups of parasitized sawfly larvae were split up into groups as indicated below.

Lot number	Approximate age of eggs at start of treatment (in days)	Number of eggs from which the capsules were not removed	Number of eggs from which the capsules were removed	Number of unencapsulated eggs found at dissection
1	9	69	74	—
2	13	42	45	46
3	15	25	30	—
4	16	14	14	—
5	22	11	12	—

Each group of eggs was placed in a separate dish of Ringer's solution and the groups were examined at intervals to determine the rate of embryonic development and hatch. The results are presented in Fig. 3.

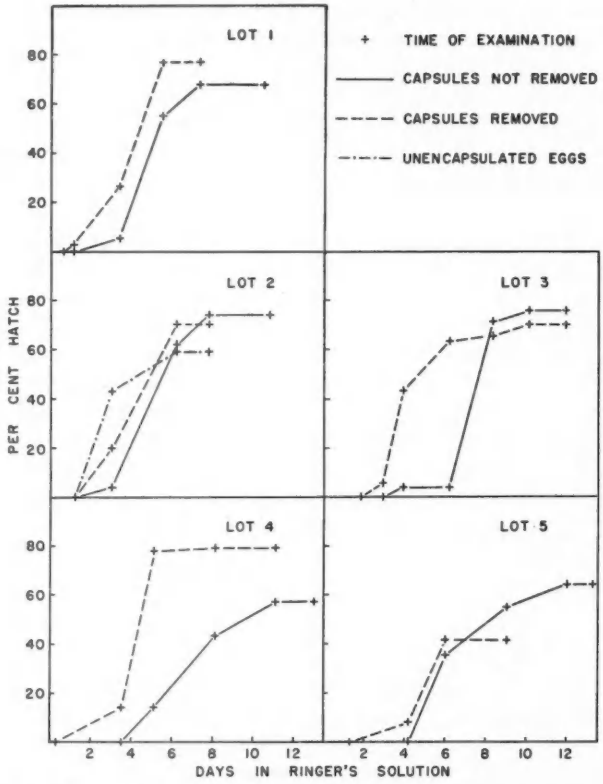

FIG. 3. The rates of hatch in Ringer's solution for (*a*) eggs with capsules left on, (*b*) eggs with capsules removed, and (*c*) unencapsulated eggs found in host larvae.

The *M. tenthredinis* eggs from which the capsules were removed developed and hatched more rapidly in Ringer's than did the eggs from which the capsules were not removed. The unencapsulated eggs found at dissection developed and hatched more rapidly in Ringer's than did the eggs from which the capsules were removed.

Effect of Time Spent in Host on Hatching Time of M. tenthredinis Eggs

The average time required for each group of eggs removed from parasitized sawfly larvae to hatch after transfer to Ringer's solution was determined. The results are given in Table IV.

The average time required to hatch in Ringer's solution at first decreased. The shortest time was required by those eggs that had spent 10–14 days in the host larvae. This is because the time required for a M. *tenthredinis* egg to hatch is approximately 7–10 days and the total time spent by a parasite egg in both the host larva and the Ringer's solution must be at least seven days.

TABLE IV

RELATION BETWEEN TIME SPENT BY *M. tenthredinis* EGGS IN LARCH SAWFLY LARVAE AND TIME REQUIRED FOR THESE EGGS TO HATCH IN RINGER'S SOLUTION

Time (in days) spent by *M. tenthredinis* eggs in sawfly larvae from parasitization to dissection	Number of eggs	Average time (in days) required for eggs to hatch in Ringer's invertebrate solution
0 to 1	50	6.1
1 to 2	217	5.2
2 to 3	71	4.6
3 to 5	102	4.3
6 to 10	373	4.0
10 to 14	336	3.8
14 to 18	96	4.6
18 to 24	53	5.9
29	66	7.0
156	40	13.0
217	35	17.0

Embryonic development of *M. tenthredinis* eggs apparently began shortly after the eggs were deposited into the sawfly larvae and continued for a period of three to four days. Then embryonic development in encapsulated eggs seemed to be abruptly inhibited. Visual observations indicated that in all cases growth was inhibited at the same stage of embryonic development. Further evidence to support this lies in the fact that those eggs which had remained in the host from 3–18 days all took approximately four days to hatch.

The data show that as the parasite embryos spent an increasingly longer time over 18 days, in the host larvae, the time required for them to hatch in Ringer's solution also increased.

Health of Host Larvae in Relation to Immunity to M. tenthredinis

A few of the groups of parasitized larch sawfly larvae dissected during this study were unhealthy. This was usually caused by failure to provide suitable cocooning media for full-grown larvae. It was occasionally due to partial starvation, and possibly excessive parasitization or disease.

Forty-two unhealthy sawfly larvae were dissected between 9 and 13 days after parasitization and it was found that 15 (or 36%) of these unhealthy larvae contained one or more parasite larvae (only 6% of the healthy sawfly larvae dissected in this study contained one or more parasite larvae). Of a total of 112 eggs deposited into these 15 unhealthy sawfly larvae that contained one or more parasite larvae, 41 (or 37%) hatched. All 71 unhatched eggs found in these 15 hosts at dissection were unencapsulated. Some of these eggs were dead when found and may have been killed by the first *M. tenthredinis* larva to hatch.

In 27 of the 42 'unhealthy' host larvae dissected, no parasite larvae were found. In these there were 103 *M. tenthredinis* eggs, of which 65 were unencapsulated, 32 were enclosed in relatively thin capsules, and 6 were enclosed

in capsules of normal thickness. Thus 37% of these 103 eggs were encapsulated as compared with 80–100% encapsulated eggs in a group of 32 apparently healthy larvae dissected at the same time after parasitization.

An analysis was carried out to determine whether host larvae that were excessively parasitized became unhealthy owing to excessive parasitization (this unhealthiness being reflected in a greater hatch of *M. tenthredinis* eggs in these host larvae). There was no definite indication that lightly parasitized host larvae inhibited the development of *M. tenthredinis* more effectively than heavily parasitized host larvae. These observations were on a relatively small scale, however, and more extensive observations might show significant trends. An analysis of the data showed that in heavily parasitized unhealthy sawfly larvae (containing 7–12 parasites) in which at least one parasite egg hatched, the number of larvae per host ranged from 2.5 to 5.0, whereas in heavily parasitized healthy sawfly larvae there were no cases where more than one larva hatched in each host.

Other Considerations

Effect of Temperature on the Immunity of the Larch Sawfly to M. tenthredinis

Dissections of larch sawfly cocoons collected from a number of areas in Manitoba from 1945 to 1951 revealed that the per cent hatch of parasite eggs followed a fairly consistent trend in each area. It was relatively higher in the odd-numbered years. An attempt was made to correlate high temperature (arbitrarily chosen as the sum of those portions of the daily maximum temperatures that were over 80° F. from July 16th to August 20th, inclusive), and low temperatures (the sum of those portions of the daily minimum temperatures under 50° F. for the same period), with the per cent hatch of *M. tenthredinis* eggs from 1945 to 1951, inclusive. A positive correlation was obtained using the high temperatures but not the low temperatures. The correlation coefficients and their significance were computed for five locations in Manitoba and one in Saskatchewan. They are:

Location	A	B	C	D	E	F
Correlation coefficient	0.56	0.57	0.37	0.85	0.40	0.68
"*t*" value	1.51	1.70	0.98	3.61	1.07	1.61
"*t*" value at the 5% level	2.57	2.45	2.45	2.57	2.45	3.18

The correlation was significant for only one location but in this case it was highly significant. Other factors that were not considered could lower the correlation and it appears noteworthy that even one high correlation was obtained.

Melanin Formation

Eckstein (6) in his investigations on the encapsulation of tachinids and ichneumonids in larvae of the sawfly *Lyda stellata* Chr. observed that melanin was formed in these capsules. He stated that this melanization was a defense reaction of the host. He and Thorpe (30) postulated that the melanin of the phagocytic capsule is formed when protein is broken down to tyrosine in the

presence of tyrosinase. Bess (2) referred to the darkening and hardening of the cells or material, or both, immediately surrounding phagocytized parasite eggs and larvae as "melanization". He concluded from his studies that "superficially, it appears that the rapidity of melanization may be a factor in causing the death of parasites in certain hosts. However, we are not certain whether melanization is a primary defense reaction or not".

In this study many of the capsules around *M. tenthredinis* eggs turned brown within larch sawfly larvae. It was assumed that these capsules had become melanized, although no chemical tests were carried out to determine whether melanin was present. The earliest brown capsules were found 12 days after parasitization in unhealthy host larvae. In most cases in apparently healthy host larvae, capsules were not darkened until at least one month after parasitization. Many of the capsules removed from sawfly larvae in February or later were darkened, but some capsules remained light-colored for a considerable period. In one case, seven out of 35 capsules removed seven months after parasitization were not darkened. Some of the host larvae dissected at this time contained both darkened and light-colored capsules.

During the period December 6th, 1951, to January 11th, 1952, *M. tenthredinis* eggs removed daily by the Forest Biology Rangers, Winnipeg, from larch sawfly larvae collected in various areas in Manitoba and Saskatchewan were divided into groups on the basis of white, light-brown, or dark-brown capsules. These groups were placed in separate lots of Ringer's solution and observed at intervals until hatching was completed. The data follow:

Color of capsule	Encapsulated eggs placed in Ringer's	Eggs hatched	Hatch, %
White	195	36	18.5
Light-brown	45	2	4.4
Dark-brown	10	0	0.0

A greater percentage of *M. tenthredinis* embryos found enveloped by white capsules at dissection were viable than were those found enveloped by brown capsules. The hatching of two of the embryos enveloped by light-brown capsules showed that browning or "melanization" of capsules can occur before the death of the *M. tenthredinis* eggs. Melanization does not seem to be the sole factor responsible for the death of the parasite embryos since only 18.5% of the embryos enclosed in apparently white capsules hatched, but it may be one of the factors that ultimately kill *M. tenthredinis* embryos within larch sawfly larvae.

Blood Count Studies

Changes in blood count in larch sawfly larvae following the introduction of tips of rose thorns were studied. Thorn tips were introduced through a proleg into larch sawfly larvae from Manitoba and British Columbia. Using tweezers, the tip of the thorn was forced into a proleg to a distance of approximately 1 mm. The protruding portion was then cut off at the integument

using scissors. The thorn tip was then pushed into the body cavity of the sawfly larva by means of a slender insect pin.

The thorns removed from larch sawfly from both Manitoba and British Columbia 6–19 days following introduction were surrounded by capsules that were similar in gross appearance to those found around eggs of *M. tenthredinis*.

Blood counts were made of 93 larch sawfly larvae from both Manitoba and British Columbia. The counts were made using a Levy counting chamber with the improved double Nebauer ruling. All counts were made on fifth-instar sawfly larvae. The following results were obtained:

Mean count of 16 untreated feeding larvae from Manitoba = 7051 cells per cu. mm. (range = 3940–14,307).

Mean count of nine untreated feeding larvae from British Columbia = 8080 cells per cu. mm. (range = 4440–15,300).

Blood counts were made on Manitoba larvae at various intervals following the introduction of thorn tips. The results are shown in Table V.

TABLE V

RELATION BETWEEN TIME SPENT BY THORN TIPS IN SAWFLY LARVAE AND THE BLOOD COUNT OF THESE LARVAE

No. of days after introduction of thorn	No. of counts made	Cells per cu. mm. (mean value)	Range of individual counts	Standard error
2	2	13,476	12,675–14,278	—
3	4	17,715	11,125–21,620	±2347
5	1	14,440	—	—
6	1	35,231	—	—
7	2	30,837	21,375–40,300	—
9	1	42,825	—	—
10	4	18,269	10,200–34,040	±5393
17	1	11,700	—	—
18	4	11,181	6420–19,225	±2924
19	1	8025	—	—

Table V shows that the number of cells per cu. mm. of larch sawfly blood increased to a maximum about nine days after introduction and then declined to about the normal concentration approximately 20 days after the thorns were introduced.

Counts were made on four sawfly larvae 10 days after the introduction of thorns. The thickness of the capsules found around these thorns related to the counts as follows:

Cells per cu. mm.	Thickness of capsule
34,040	Very thin
16,075	Thin
12,760	Medium sized
10,200	Relatively thick

Ten days after the introduction of thorns the thinnest capsules were found on the thorns removed from the larvae having the highest concentration of cells.

Discussion

In the preceding section it was stated that larch sawfly larvae from British Columbia possess the ability to form capsules around thorn tips. It was also observed that 'foreign bodies' found within sawfly larvae from British Columbia at dissection were usually encapsulated. On the other hand, it will be recalled that eggs of *M. tenthredinis* were rarely encapsulated in larvae from British Columbia. Moreover, when superparasitism occurred in larch sawfly larvae from British Columbia the dead *M. tenthredinis* embryos and first-instar larvae were not encapsulated. These observations indicate that the phagocytes of the sawfly larvae from British Columbia do not encapsulate *M. tenthredinis* even when this parasite is unhealthy or dead and in all probability giving off 'pathological exudates'. Furthermore in no instances were the first-instar *M. tenthredinis* larvae found in larch sawfly from Manitoba and Saskatchewan encapsulated. This was true for both living and dead parasite larvae.

According to the hypothesis that the phagocytes are not a primary factor in immunity but are attracted only to unhealthy parasites and degenerating cells it would have to be assumed that internal conditions in the Manitoba–Saskatchewan larch sawfly differ from those of the British Columbia larch sawfly to the extent that *M. tenthredinis* eggs become unhealthy in the former hosts but not in the latter. This still would not explain why dead or dying *M. tenthredinis* first-instar larvae were not encapsulated in either host.

A second explanation might be that *M. tenthredinis* eggs remain healthy after deposition and offer identical stimuli during development to the phagocytes of both types of host but phagocytes of the sawfly from British Columbia may not be able to respond to these stimuli whereas the phagocytes in the sawfly from Manitoba and Saskatchewan are able to do so.

A third possibility is that *M. tenthredinis* eggs secrete a substance that is able to immobilize the phagocytes of the sawfly larvae from British Columbia but not those of the sawfly larvae from Manitoba and Saskatchewan. The thorns that were encapsulated in sawfly larvae from British Columbia were all introduced into unparasitized larvae. Also, the encapsulated 'foreign bodies' found on dissection in certain sawfly larvae from British Columbia were all found in unparasitized sawfly larvae. To explore this hypothesis, further experiments should be carried out to introduce thorns into sawfly larvae from British Columbia shortly after parasitization by *M. tenthredinis* to determine whether these larvae still retain the ability to deposit capsules about the thorns.

Occasionally, where *M. tenthredinis* larvae were found in larch sawfly from Manitoba and Saskatchewan, encapsulated *M. tenthredinis* eggs were also found in the same host larvae. In a few instances, empty, encapsulated chorions were found along with living first-instar *M. tenthredinis* larvae. These observations imply that the immunity mechanism sometimes breaks down for particular encapsulated *M. tenthredinis* embryos. The view that successful *M. tenthredinis* embryos immobilize the phagocytes does not apply in this case.

Nevertheless, in the author's opinion, the weight of evidence obtained favors the interpretation that the phagocytes of the larch sawfly in Manitoba and Saskatchewan play an important role in the immunity reaction of this insect to the parasite *M. tenthredinis*.

Summary and Conclusions

Unless otherwise stated, conclusions refer to host material from Manitoba and Saskatchewan.

1. In a collection of 53 British Columbia larch sawfly larvae parasitized by *M. tenthredinis*, the parasite developed normally in 51 (or 96%) and in these hosts no encapsulation occurred. In 2 (or 4%) of these 53 larvae development of the parasite was arrested and these hosts contained encapsulated eggs. In contrast to this situation, *M. tenthredinis* develops normally in relatively few hosts in Manitoba and Saskatchewan where a high degree of encapsulation of *M. tenthredinis* eggs occurs.

2. No relation was found between the larch sawfly larval stage attacked and the effective parasitism by *M. tenthredinis*. No relation was found between the seasonal period of attack and the effective parasitism by *M. tenthredinis*.

3. The percentage of *M. tenthredinis* eggs found encapsulated by phagocytes at dissection increased as the time spent by these eggs in sawfly larvae increased. Almost 100% of the parasite eggs were encapsulated after they had spent about 10 days in healthy host larvae.

4. A number of the encapsulated *M. tenthredinis* eggs removed from host larvae hatched after they were transferred to Ringer's solution. This was true whether or not the capsules were removed from these eggs before they were placed in the solution.

5. *M. tenthredinis* eggs, from which the capsules were removed, developed and hatched more rapidly in Ringer's solution than eggs from which the capsules were not removed. Unencapsulated eggs found at dissection developed and hatched more rapidly in Ringer's solution than eggs from which the capsules were removed.

6. Embryonic development of *M. tenthredinis* eggs began shortly after they were deposited into the host larvae and continued for a period of three to four days at which time growth in encapsulated eggs was abruptly inhibited.

7. The length of time required for eggs of *M. tenthredinis* to hatch in Ringer's solution is related to the length of time they remained in the host larvae. The average time required for the parasite eggs to hatch in Ringer's at first decreased as the time spent by the eggs in the sawfly larvae increased. This is due to the fact that the first part of the parasite's embryonic development occurs in the host larva. As encapsulated *M. tenthredinis* embryos spent an increasingly longer time over 18 days in the host larvae, the time required for them to hatch in Ringer's solution increased.

8. The longer the encapsulated *M. tenthredinis* eggs remained in the host larvae, the lower was the percentage that hatched in Ringer's solution. Viable parasite embryos were found in host larvae seven months after the eggs were deposited. The factor or factors responsible for preventing the *M. tenthredinis*

eggs from developing and hatching in the larch sawfly did not kill the parasite eggs immediately but inhibited growth and differentiation of the embryos.

9. A greater percentage of *M. tenthredinis* eggs hatched in unhealthy host larvae. Fewer parasite eggs were encapsulated in unhealthy host larvae than in healthy host larvae. There was no indication that lightly parasitized larvae inhibited development of *M. tenthredinis* more effectively than heavily parasitized larvae.

10. A fairly consistent yearly correlation in trend was obtained between high summer temperatures and the per cent hatch of *M. tenthredinis* eggs. Higher summer temperatures appeared to result in a higher per cent hatch of *M. tenthredinis* eggs.

11. A greater percentage of the *M. tenthredinis* embryos found enveloped by white capsules at dissection were viable than were those found enveloped by brown capsules. Because some eggs found enclosed in light brown capsules at dissection were viable, browning or "melanization" of capsules can occur before the death of *M. tenthredinis* eggs and may be one of the factors that ultimately kill the embryos within host larvae.

12. Sawfly larvae from both Manitoba and British Columbia possessed the ability to deposit capsules about the thorn tips introduced into their bodies.

13. The number of hemocytes per cu. mm. of larch sawfly blood increased to a maximum at about nine days after the introduction of thorns and then declined to about the normal concentration approximately 20 days after the thorns were introduced. Ten days after the introduction of thorns the thinnest capsules were found on the thorns removed from larvae having the highest concentration of hemocytes.

14. It is concluded that the evidence favors the interpretation that the phagocytes of the larch sawfly in Manitoba and Saskatchewan play an important role in the immunity of this insect to *M. tenthredinis*.

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COMPARATIVE TRANSMISSION OF POTATO VIRUS Y BY FOUR APHID SPECIES THAT INFEST POTATO[1]

By R. H. E. Bradley[2] and D. W. Rideout[2]

Abstract

Single aphids of four species were observed with a hand-lens until each had touched its proboscis once on a tobacco plant infected with potato virus Y and then once on a healthy tobacco plant. The time that the proboscis remained touching the plants in each case was recorded. This procedure required only a few minutes for each aphid, and sometimes it was completed in less than a minute. During this process, the successful transmissions of potato virus Y by single aphids of *Myzus persicae* (Sulz.), *Aphis abbreviata* Patch, *Macrosiphum solanifolii* (Ashm), and *Myzus solani* (Kltb.) were 55, 31, 9, and 4% respectively. Vector efficiency was not due to difference in feeding behavior of these species. When single infective aphids were transferred to a series of five plants at five-minute intervals, *M. persicae* caused more infections and remained infective longer than *A. abbreviata*.

Introduction

Aphid species vary in their abilities to transmit potato virus Y (1, 4, 11), and this is generally true of aphid-transmitted viruses. However, the results of different workers who have investigated the vector efficiencies of aphid species often cannot be compared; and in the light of present knowledge some results have lost their significance. Until about 15 years ago, large numbers of aphids that had fed for several hours or days on infected plants were used to transmit viruses like those that cause cucumber mosaic, sugar beet mosaic, and western celery mosaic; and transmissions were not always successful. With such methods, Severin and Freitag (5) transmitted western celery mosaic virus with 11 species of aphids, and the percentage of transmission obtained varied from 14 to 85. Watson (9, 10) and Watson and Roberts (11) showed that henbane mosaic, potato Y, cucumber mosaic, and sugar beet mosaic viruses were transmitted much more efficiently when the vectors were first fasted for about an hour and then placed on infected plants for only a few minutes. This change in procedure made it possible to obtain a high percentage of transmissions, with single vectors, of most of the aphid-transmitted viruses that cause mosaic symptoms in their hosts.

Myzus persicae (Sulz.) usually acquires sugar beet mosaic (7), potato Y (3), and henbane mosaic (2) viruses in less than a minute on infected plants, and can immediately infect healthy plants during equally brief periods; the time that the proboscis touches the plants during these brief periods affects the probability of transmission. Therefore, the feeding behavior of an aphid species during transmission of such viruses may largely be responsible for its efficiency as a vector. To test this hypothesis, the authors studied the vector efficiencies and feeding behaviors of four aphid species transmitting potato virus Y.

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Materials and General Methods

The species of aphids used were the green peach aphid, *M. persicae;* the buckthorn aphid, *Aphis abbreviata* Patch; the potato aphid, *Macrosiphum solanifolii* (Ashm.); and the foxglove aphid, *Myzus solani* (Kltb.) [= *Myzus convolvuli* (Kltb.)]. These four species infest potatoes in Eastern Canada, but *M. solani* is rarely abundant in the presence of the other species. At first, aphids were collected from potatoes in the field, but during the latter half of the work they were reared on potato plants in the greenhouse. No differences were observed in the feeding behaviors and vector efficiencies of the aphids from these two sources, and therefore all of the results are considered together. In most of the experiments, several groups of 5 to 10 aphids of each species were transferred directly from the potato plants to healthy tobacco plants. None of these plants developed symptoms, which shows that the aphids were not carrying viruses that would infect tobacco. Furthermore, large numbers of healthy tobacco plants were kept in the greenhouse during all experiments, and not one accidental infection was found.

The methods of collecting and handling the aphids and the strain of potato virus Y (PVY) have already been described (3). Usually, only wingless adults were used, but occasionally it was necessary to include a few third- or fourth-instar nymphs. All transmissions were to and from White Burley tobacco (*Nicotiana tabacum* L.). The source of infection for aphids was aging leaves from medium-aged tobacco plants showing well-developed symptoms of infection with PVY. Aphids were tested for infectivity on young healthy tobacco plants in the three- to five-leaf stage. The plants were covered with plain drinking tumblers during the test-feeding period to prevent aphids from infesting the greenhouse. The tumblers were removed after the test-feeding period, and the plants fumigated with nicotine to kill the aphids.

To obtain a high efficiency of transmission, all of the aphids were kept without food from one to four hours before they were placed on the source of infection (11). When placed on plants after this period of preliminary fasting, each aphid usually touched its proboscis to the leaf, as if inserting its stylets, for a few brief periods before it settled down to continuous feeding. These brief attempts to feed will be referred to as *feeding punctures* rather than feeding periods, because it is not known whether aphids imbibe food during such short intervals. *Infection-feeding punctures* are those made on a source of infection, and *test-feeding punctures* are those made on the test plants. The feeding behavior during transmission of PVY was followed by carefully observing single aphids with a hand-lens for varying periods on infected and healthy plants. The time that each aphid wandered before attempting to feed was noted, and the time that the proboscis remained touching the leaf was recorded as 5-15, 16-30, 31-60, or 61-120 sec. When the feeding puncture lasted over 120 sec., the aphid was disturbed at 121 sec. with a camel's-hair brush.

Feeding Behaviors and Vector Efficiencies

The feeding behaviors and the efficiencies with which the four species of aphids transmitted PVY were compared in the first experiment. Single aphids were permitted one infection-feeding puncture each and then transferred to two successive test plants. Each aphid was observed until it had completed one test-feeding puncture on the first test plant, and then it was transferred to the second test plant for 24 hr. The durations of the infection-feeding punctures and the initial test-feeding punctures were recorded. Five aphids of each species were tested on 30 occasions. This made a total of 150 aphids of each species except *M. solani*, which was available for only 10 of the experiments.

Aphids of these four species required only a few minutes to transmit PVY from infected to healthy plants. The chief difference observed in the feeding behavior during transmission was that after an hour of fasting *M. persicae* and *A. abbreviata* nearly always made feeding punctures as soon as they were placed on the plants, whereas *M. solanifolii* and *M. solani* often would not attempt to feed. A greater number of *M. solanifolii* and *M. solani* would attempt to feed after three or four hours of fasting, but longer periods of fasting did not change their behavior. In most of the experiments, many of *M. solanifolii* and *M. solani* had to be rejected because they did not attempt to feed.

The durations of the infection-feeding punctures and those of the initial test-feeding punctures of *M. persicae*, *A. abbreviata*, and *M. solanifolii* were very similar (Table I). The briefest feeding punctures lasted about eight seconds. Over 70% of the infection-feeding punctures lasted less than 30 sec., and about 10% lasted over a minute. In comparison, about 50% of the initial test-feeding punctures lasted less than 30 sec., and about 20% lasted over a minute. Over twice as many aphids were disturbed at 121 sec. during the initial test-feeding puncture as during the infection-feeding puncture. This would be expected because each aphid usually makes a few brief feeding punctures before it settles down to continuous feeding, and more aphids would settle down during the second feeding puncture than during the first.

The duration of the infection-feeding puncture appeared to have no effect on the duration of the initial test-feeding puncture. The probability that the initial test-feeding puncture would last over a minute was about the same when the infection-feeding puncture lasted less than a minute as when it lasted over a minute.

Table I shows that 39 of *M. persicae* made infection-feeding punctures lasting 5-15 sec. Twenty-nine of these aphids, or 74%, transmitted PVY. The percentages of aphids of each species that transmitted PVY after the infection-feeding punctures in Table I are shown in Fig. 1. The effect of the duration of the infection-feeding puncture on the percentage of transmissions was similar for each species. Regardless of the duration of the infection-feeding puncture, up to two minutes, *M. persicae* consistently transmitted the virus more often than *A. abbreviata*, which in turn consistently did so more

TABLE I

DURATIONS OF INFECTION-FEEDING PUNCTURES AND INITIAL TEST-FEEDING PUNCTURES OF THREE SPECIES OF APHIDS (150 APHIDS OF EACH SPECIES)

Species	Infection-feeding puncture, sec.					Initial test-feeding puncture, sec.				
	5-15	16-30	31-60	61-120	Disturbed at 121	10-15	16-30	31-60	61-120	Disturbed at 121
M. persicae	39	63	33	10	5	37	49	28	17	19
A. abbreviata	42	65	25	8	10	34	57	26	21	12
M. solanifolii	53	66	14	11	6	42	44	29	7	28
Total	134	194	72	29	21	113	150	83	45	59
%	30	43	16	6	5	25	33	19	10	13

often than *M. solanifolii*. In each case the highest percentage of transmission was effected by those aphids that made infection-feeding punctures lasting 16-60 sec. Of the 21 aphids disturbed at 121 sec., only four transmitted PVY. Though the percentages of transmissions by the disturbed aphids of each species would not be accurate, the results indicate that the disturbed aphids transmitted virus only about half as often as the aphids that completed the infection-feeding puncture in less than two minutes.

The percentages of aphids that caused infection during the initial test-feeding punctures in Table I are shown in Fig. 2. The curves in Fig. 2 are similar in shape to those in Fig. 1; and the effect of the duration of the initial

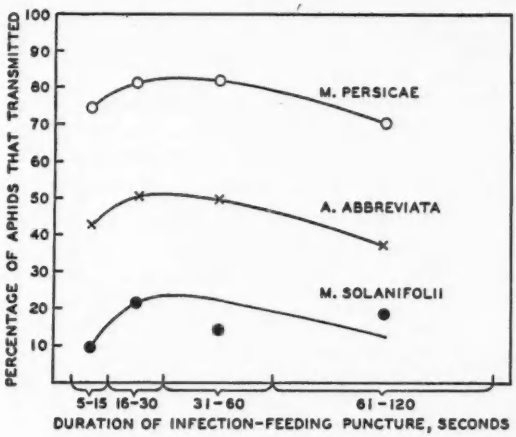

FIG. 1. Percentages of aphids that transmitted potato virus Y after the infection-feeding punctures in Table I.

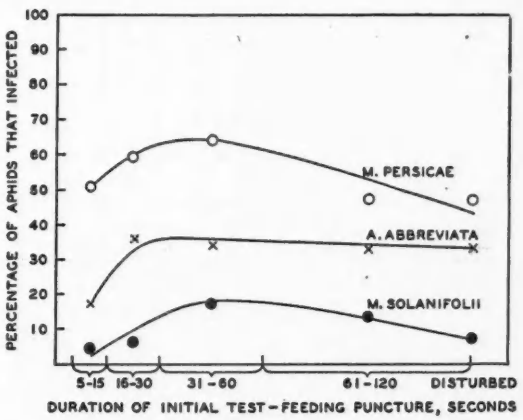

FIG. 2. Percentages of aphids that infected the first test plant with potato virus Y during the initial test-feeding punctures in Table I.

test-feeding puncture on the probability of infection was similar for each species. Aphids caused infection during test-feeding punctures that lasted less than 15 sec., but there was an increase in the effectiveness of test-feeding punctures up to 31-60 sec. There was a small drop in the effectiveness of test-feeding punctures that lasted over a minute. Regardless of the duration of the initial test-feeding puncture, up to two minutes, *M. persicae* consistently caused infection more often than *A. abbreviata*, which in turn did so more often than *M. solanifolii*. The average number of plants infected by *M. persicae*, *A. abbreviata*, and *M. solanifolii* during the initial test-feeding puncture was 55, 31, and 9% respectively.

M. solani was included in 10 replications of this experiment, and only two out of 50, or 4%, transmitted PVY. The behavior of this species closely resembled that of *M. solanifolii*, and the durations of the feeding punctures of *M. solani* were similar to those of the other species. The two of *M. solani* that transmitted PVY infected the first test plant only. One of these acquired PVY during an infection-feeding puncture that lasted less than 15 sec., and then transmitted the infection during a test-feeding puncture that lasted less than 15 sec. The other became infective during an infection-feeding puncture that lasted about a minute, and infected during a 31-60 sec. test-feeding puncture. These results show that *M. solani* is less efficient than *M. solanifolii* as a vector of PVY.

TABLE II

NUMBERS OF FIRST AND SECOND TEST PLANTS INFECTED BY THREE APHID SPECIES. (EACH APHID MADE ONE FEEDING PUNCTURE ON FIRST PLANT AND WAS LEFT 24 HR. ON SECOND PLANT; 150 APHIDS OF EACH SPECIES)

Species	Test plants infected				Aphids infective out of 150	% infective
	Both	First	Second	Neither		
M. persicae	56	27	32	35	115	77
A. abbreviata	16	31	21	82	68	45
M. solanifolii	6	7	12	125	25	17

Some of the aphids failed to cause infection during the initial test-feeding puncture but infected the second test plant, on which they fed for 24 hr. The results of infections of both test plants are shown in Table II. The overall efficiency of transmission of PVY by single aphids of *M. persicae*, *A. abbreviata*, and *M. solanifolii* after one infection-feeding puncture was 77, 45, and 17% respectively. Of the aphids that transmitted PVY, about 50% of *M. persicae* and 25% of *A. abbreviata* and *M. solanifolii* infected both plants. It can also be calculated from Table II that the percentages of *M. persicae*, *A. abbreviata*, and *M. solanifolii* that infected the first plant were 72, 69, and 52 respectively: and the percentages that infected the second plant were

77, 54, and 72 respectively. These results show that the likelihood of infection by these species during the initial test-feeding puncture was lowest for *M. solanifolii;* but the likelihood of infection after the initial test-feeding puncture was lowest for *A. abbreviata.*

Loss of Infectivity by M. persicae and A. abbreviata

In this experiment, single aphids of *M. persicae* and of *A. abbreviata* were transferred after one infection-feeding puncture to a series of five test plants at five-minute intervals. Each aphid remained overnight on the fifth test plant. Aphids of both species usually made at least one feeding puncture during the five-minute intervals. Five aphids of each species were tested on 10 occasions; and out of 50 aphids, 41 of *M. persicae* and 18 of *A. abbreviata* transmitted PVY. The numbers of plants infected by each aphid are shown in Table III. Twelve of *A. abbreviata* infected one plant each and the remaining six infected two plants. In comparison, the percentages of *M. persicae* that infected one, two, three, four, and five plants of each series were 34, 37, 17, 10, and 2 respectively. The average number of plants infected by each

TABLE III

INFECTIONS BY SINGLE ADULTS OF *M. persicae* AND *A. abbreviata* WHEN TRANSFERRED TO FIVE TEST PLANTS AT FIVE-MINUTE INTERVALS. (EACH APHID REMAINED OVERNIGHT ON FIFTH PLANT)

Numbers that infected out of a total of 50 of each species		Test plant infected	Total plants infected by each aphid
M. persicae	*A. abbreviata*	A B C D E	
11	10	x* – – – –	
1	1	– x – – –	
	1	– – x – –	1
1		– – – x –	
1		– – – – x	
13	5	x x – – –	
1		x – x – –	2
	1	x – – – x	
1		– x – – x	
4		x x x – –	
1		x x – x –	3
1		– x x x –	
1		– – x x x	
1		x x x x –	
1		x x – x x	4
2		x x x – x	
1		x x x x x	5
Total 41	18		

* x, *plant infected;* –, *plant not infected.*

aphid that transmitted was 2.1 for *M. persicae* and 1.3 for *A. abbreviata*. These results show that *M. persicae* infected more plants and remained infective longer than *A. abbreviata*.

Discussion

The main difference observed in the feeding behavior of these species during transmission of PVY was that many of *M. solanifolii* and *M. solani* often did not attempt to feed after a period of fasting; but this would not account for the large differences in vector efficiency in these experiments, because only aphids that were observed to attempt to feed were used. The failure of many of *M. solanifolii* and *M. solani* to attempt to feed may, however, lower the vector efficiencies of these species in the field, and probably in experiments where the aphids are not observed individually.

The period that the proboscis of each aphid touched the plants during transmission was similar for each species (Table I), but the likelihood of infective aphids causing infection during a single feeding puncture was greater for *M. persicae* and *A. abbreviata* than for *M. solanifolii* (Table II). The stylets of *M. persicae* usually pass intercellularly, whereas those of *M. solanifolii* usually pass intracellularly (6). Also, during brief feeding punctures, the stylets of *M. persicae* pass only superficially into the epidermal cells, and little or no saliva is ejected (2). It is possible that the manner of stylet penetration differs with each species, and such differences may largely be responsible for vector efficiency. Further investigations along this line are in progress.

The vector efficiency of a species appears to depend on more than one factor. In addition to variation in the probability of infection during a feeding puncture, species vary in the time they remain infective, and in the number of times they can infect (Table III). Therefore, successful transmission of a virus from an infected to a healthy plant is not the only criterion of vector efficiency. For example, other factors being equal, infective *M. persicae* are a greater potential danger to a crop than are infective *A. abbreviata*.

These experiments are the first to be described in which single adults of *M. persicae*, *A. abbreviata*, *M. solanifolii*, and *M. solani* are compared as vectors of PVY, and the results show differences in vector efficiency that were not previously recognized. Bawden and Kassanis (1) obtained nearly 100% transmission with each of *M. persicae*, *M. solanifolii*, and *Aphis rhamni* B. de F. [*A.* ? *abbreviata* Patch] when 10 aphids were fed on each test plant. Such a result would be expected, because in the present experiments 17% transmission was obtained with single adults of *M. solanifolii*, and this was the least efficient vector of the above species (Table II). Kassanis (4) reported that *M. persicae* and *Aphis rhamni* B. de F. [*A.* ? *abbreviata* Patch] were equally efficient vectors of PVY under experimental conditions; but he also tested groups of aphids and obtained nearly 100% transmission with both species. In the present results, nearly twice as many transmissions were obtained with *M. persicae* as with *A. abbreviata* (Tables II and III). The

results of different workers who compare the vector efficiencies of aphid species would be of greater value if the numbers of aphids tested on each plant were so reduced that less than 100% transmission is obtained in all cases.

The four species of aphids that infest potatoes in Eastern Canada can infect plants with PVY in less than a minute. Therefore, insecticides would not prevent winged vectors from introducing PVY into a crop unless winged migrants were killed soon after they alight and before they attempt to feed. Furthermore, spread of PVY within a field may be increased if insecticides cause the aphids to move from plant to plant attempting to feed before they die.

Acknowledgments

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SOME EFFECTS OF A FOLIC ACID ANTAGONIST ON THE RESPONSES OF THE IMMATURE PULLET TO TREATMENT WITH GONADAL HORMONES[1]

By W. E. J. Phillips[2], W. A. Maw[3], and R. H. Common[4]

Abstract

Aminopterin was found to depress the hypertrophy of the oviduct of immature pullets induced by estrogen plus androgen. The diet was arranged to be low in folic acid during the experimental period. Higher dosage levels of aminopterin did not further decrease the size of oviduct in estrogenized pullets below a plateau level which was higher than that of the unestrogenized controls. Aminopterin opposed the effects of the hormonal treatment on liver weight per kgm. live weight, adrenal weight, and spleen weight. Injections of folic acid not merely antagonized this effect of aminopterin, but increased the size of the spleen above that of the estrogenized controls. Intraperitoneal injections of desoxyribonucleic acid also opposed the effect of aminopterin on the spleen of the estrogenized pullet. Aminopterin appeared to have no effect on liver phosphoprotein P of the estrogenized pullet.

Neither aminopterin nor aminopterin plus desoxyribonucleic acid affected serum calcium level significantly, but aminopterin plus folic acid significantly lowered the serum calcium level of the estrogenized pullet in one experiment. The differences between the groups in respect of liver nucleic acid content were slight and did not attain statistical significance.

Introduction

Hertz (5) has demonstrated that dietary deficiency of folic acid will limit the response of the chick's oviduct to estrogen, and that the response is also limited by administration of the folic acid antagonist aminopterin. Hertz also demonstrated that the effects of aminopterin could be reversed by administration of sufficient folic acid. Hertz and Tullner (7) subsequently worked out the quantitative relationships of folic acid and aminopterin in regard to inhibition of the response of the oviduct to estrogen. Kline and Dorfman (8) found that citrovorum factor can oppose the inhibitory effect of aminopterin on the response of the chick's oviduct to stilbestrol. Snell and Cravens (13) have shown that injection of aminopterin into the egg before incubation, or during the early stages of incubation, was highly toxic to the embryo. However, injection of thymidine partially counteracted the effects of aminopterin, a combination of thymidine and hypoxanthine desoxyriboside being more effective than thymidine alone. These observations supported the view that aminopterin exerts its effect by inhibiting synthesis of one or more of the purine desoxyribosides. Aureomycin (16) and citrovorum factor (3) have also been shown to be effective in overcoming the toxic effects of aminopterin. It may be mentioned here that folic acid antagonists have been found not to interfere with the action of testosterone propionate on the

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combs and testes of cockerels (17). This suggests that interference with nucleic acid synthesis may not prejudice hypertrophy of these organs, although it prejudices the response of the oviduct.

The present paper reports studies on the inhibitory effects of aminopterin on the growth of the oviduct of the sexually immature pullet, and on the effect of certain substances in overcoming this inhibition.

Experimental

Experiment I

General Technique and Analytical Methods

The pullets (New Hampshire male × Barred Plymouth Rock female) were housed in separate cages and were approximately 10 weeks of age at the beginning of the experiment. The basal diet was designed so as to be low in folic acid, and was made up as follows:—

Maize meal	35 lb.	Fish liver oil (2000 units A and 300 units D per gram)	0.25 lb.
Ground wheat	16.5 "		
Ground oats	10 "		
Ground barley	25 "	Manganese sulphate	11.4 gm./100 lb.
"Vitamin free" casein	10 "	Niacin	0.5 gm./100 lb.
Limestone flour	2 "	Riboflavin	3.0 gm./100 lb.
Rock phosphate	1 "	Calcium pantothenate	0.25 gm./100 lb.
Common salt	0.5 "		

Twenty-six pullets were placed in individual cages and given this diet for a week until they had become thoroughly accustomed to their new surroundings. Food consumption was maintained at 70 gm. per day. The birds were then placed on experiment. The treatments are reported in Table I; here it should be pointed out that Group 1 served as negative controls, and Group 2 as a positive control group receiving hormones only. All the other birds except Group 6 (*vide infra*) were treated with aminopterin (4-aminopteroylglutamic acid, kindly presented by Dr. T. H. Jukes, Lederle Laboratories), successive groups receiving progressively increasing amounts. The aminopterin was suspended in aqueous solution and administered by subcutaneous injection. The aminopterin treatments were given daily for three days before the hormonal treatments in order to ensure distribution of the antagonist throughout the systems of the birds before superimposing other treatments (8). The aminopterin treatments were continued daily thereafter. (The experiment was originally planned on an exploratory basis, and the 12 birds of Groups 3 to 5 were originally arranged in seven groups of one or two birds each receiving progressive dosage levels of 0.1, 0.25, 0.5, 1.0, 2.0, 3.0, and 3.0 mgm. aminopterin. However, inspection of the detailed results suggested that if the birds receiving aminopterin treatments were re-grouped as in Table I, then the data might be interpreted on a statistical basis. This, as will be seen below, proved to be correct.)

It was known that aminopterin at the larger dosage levels, i.e. greater than about 0.5 mgm. daily, will reduce appetite. Since responses to hormonal treatment may be influenced by food intake, Group 6 was maintained as an

inanition control group by reducing the food intake step by step so that the total food intake would approximate that of the birds receiving the higher levels of aminopterin. Inclusion of an inanition control has been found essential in such experiments.

The hormonal treatments were begun on the fourth day of experiment, and comprised the intramuscular injection of appropriate amounts (*vide* Table I) of estradiol benzoate, (Progynon B, Schering) and testosterone propionate (Oreton, Schering) on five alternate mornings. The birds were killed and dissected for analysis on the second morning after the last injection.

The preparation of the livers for analysis has been described previously (2, 11). Pentose nucleic acid (RNAP) and desoxypentose nucleic acid (DNAP) were determined on the tissue powders by the method of Schneider (12).

Serum calcium was determined in a trichloroacetic acid filtrate of serum by Halverson's method as described by Peters and Van Slyke (10). Direct precipitation of oxalate from serum is inapplicable to sera of laying or estrogenized birds because of interference by serum lipophosphoprotein.

The method for liver phosphoprotein was adopted in the light of Davidson's (4) observation that the residue of protein left after extraction of nucleic acids by hot trichloroacetic acid contains phosphorus other than phosphoprotein phosphorus. One hundred milligrams of liver tissue powder was subjected to extraction of the nucleic acids by hot 5% trichloroacetic acid in the usual way. The residue in the centrifuge tube was then incubated with 5 ml. of 1.0 N potassium hydroxide for 15 hr. at 35° C. The alkaline extract was cleared by centrifuging at 3000 r.p.m. for 10 min. and then decanted into a 10 ml. graduated cylinder. The residue was stirred with 5 ml. of 1.0 N potassium hydroxide and again centrifuged. The supernatant was added to the original extract and the volume made up to 10 ml. with 1.0 N potassium hydroxide. This alkaline extract contained the phosphoprotein phosphorus as inorganic phosphate, but since it also contained other phosphorus compounds, this inorganic phosphate could not be determined by determining the total P of the alkaline extract. Accordingly, the method of Martin and Doty (9) for determining inorganic phosphate in the presence of organic phosphorus was used.

Experimental Results

Experiment I

The experimental results are presented in Table I.

The control groups and the birds receiving amounts of aminopterin up to 0.25 mgm. per day appeared to remain in excellent condition. Those receiving 0.5 mgm. per day or more were unkempt, failed to preen, had dirty beaks, and kept their heads retracted, while their droppings were green.

Live weight gain during the experiment decreased with increasing dosage levels of aminopterin. The decrease was related in all likelihood to a decrease in feed consumption, and the live weight gains of the inanition controls provided confirmation on this point.

TABLE I

EFFECTS OF GRADED DOSES OF AMINOPTERIN ON CERTAIN RESPONSES OF THE IMMATURE PULLET TO ESTROGEN PLUS ANDROGEN. TOTAL DURATION OF EXPERIMENT, 13 DAYS; HORMONAL TREATMENTS ADMINISTERED ON ALTERNATE DAYS OVER LAST 10 DAYS OF EXPERIMENT

Group	1 Negative control	2 Positive control	3 Aminopterin	4 Aminopterin	5 Aminopterin	6 Inanition control	
No. of pullets	4	4	4	4	4	2	
Treatment:							L.S.D.*
Hormone treatment:							
ODB, mgm.	Nil	5 × 1.67	5 × 1.67	5 × 1.67	5 × 1.67	5 × 1.67	$P = 0.05$
TST, mgm.	Nil	5 × 0.8	5 × 0.8	5 × 0.8	5 × 0.8	5 × 0.8	
Aminopterin treatment: mgm. daily	Nil	Nil	0.1 to 0.25	0.5 to 2.0	3.0 to 4.0	Nil	
Live weight, initial kgm.	0.82	0.79	0.82	0.77	0.88	0.86	
Live weight, final kgm.	1.08	1.09	1.00	0.78	0.69	0.80	
Live weight, gain kgm.	0.26	0.30	0.18	0.01	−0.19	−0.06	
Feed consumption kgm.	0.69	0.69	0.61	0.41	0.25	0.30	
Liver, gm.	22.1	31.8	30.0	19.5	17.1	18.7	
Liver, gm./kgm. live weight	20.5	29.0	30.2	25.1	25.3	23.4	
Oviduct, gm.	0.12	11.8	4.4	2.5	2.4	12.1	1.72
Serum Ca, mgm./100 ml.	13.4	61.9	48.2	72.6	59.3	73.7	13.4
Liver DNAP, mgm./100 gm.	27.0	21.3	20.9	21.8	23.8	26.0	
" " mgm./kgm. live weight	5.5	6.1	6.3	5.4	5.5	6.1	N.S.
Liver RNAP, mgm./100 gm.	67.3	52.2	49.2	62.2	65.5	84.5	
" " mgm./kgm. live weight	13.5	15.0	14.8	15.8	16.5	19.7	N.S.
Ratio $\frac{\text{RNAP}}{\text{DNAP}}$	2.50	2.49	2.37	2.84	2.83	3.27	N.S.
Liver PPP, mgm./100 gm.	1.81	5.24	4.83	7.10	5.78	6.84	
Liver PPP, mgm./liver	0.40	1.67	1.45	1.39	1.07	1.28	
Liver PPP, mgm./kgm. live weight	0.37	1.5	1.5	1.8	1.6	1.6	0.50

* *The analysis of variance did not include the data for the two birds of the inanition control group.*

The hormonal treatment evoked the expected large increase in liver weight and weight of liver per kgm. live weight (comparison of Groups 1 and 2). This increase was not affected by aminopterin until a dosage of 0.5 mgm. per day was reached and passed. The subsequent decrease was probably related to decreased feed consumption, for the inanition control group did not display so great an increase in weight of liver per kgm. live weight. There was not any evidence that aminopterin reduced the hypercalcaemia induced by the hormonal treatment.

The results for oviduct weights are of interest. The increase of oviduct weight evoked by the hormonal treatment was progressively smaller with increasing dosage levels of aminopterin. However, no further suppression of the hypertrophy evoked by the hormones was attained by increasing the level of aminopterin beyond 0.5 mgm. per day.

Hertz, Larsen, and Tullner (6) have presented data which demonstrate the effect of progressively increasing levels of progesterone in suppressing hypertrophy of the oviduct evoked by estrogen. There is a striking analogy between these data and those now reported for the effect of aminopterin on hormonally induced hypertrophy of the oviduct. Beyond a certain dosage level, neither progesterone nor aminopterin will further suppress the degree of hypertrophy. If the values reported by Hertz, Larsen, and Tullner (6) are expressed as the ratio:

$$\frac{\text{Wt. of oviduct from estrogen only}}{\text{Wt. of oviduct from treatment with estrogen + progesterone}}$$

then a maximal value of 4.2 is approached. If the values in the present experiment are expressed in an analogous way, then a ratio of 4.7 is obtained. Hence two different antagonists, presumably acting in different ways, and acting on birds of different ages, have given approximately the same limiting degree of suppression of stimulation of the oviduct by estrogen.

The present results provide some further evidence that the growth of the oviduct induced by estrogen or by estrogen plus androgen is made up of two distinct components. One of these can be suppressed by the antagonists, the other cannot be thus suppressed. The data for the inanition controls suggest that the effect of aminopterin cannot be attributed solely to the effect on appetite, the size of the oviducts in this group being of the same order as those of the positive control group.

Neither the results for liver DNAP nor those for RNAP display significance of treatments effects. Nevertheless, the trends of the averages are sufficiently consistent to suggest that the differences may be real though not significant, and that further work might establish significance. Therefore, the results cannot be advanced in support of the view that the treatments did not affect the liver nucleic acids, and they may be discussed as pointing the way to further experimentation. The trends of the averages suggest (*a*) that the hormonal treatment had produced its known effect of increasing liver DNAP per kgm. live weight; (*b*) that aminopterin opposed this effect of the hormonal

treatment; and (*c*) that the effect of aminopterin was not due to inanition per se. The trends of the averages for RNAP suggest that (*a*) the hormonal treatments increased liver RNAP per kgm. live weight, though to a smaller extent than might have been expected; (*b*) that aminopterin did not oppose this effect even where there was severe inanition. If the significance of these effects were to be established by further experimentation, then the data would provide support for the hypothesis that aminopterin affected the response to gonadal hormones by interfering with synthesis of desoxyribonucleic acid, perhaps by interference with thymidine synthesis.

The hormonal treatment increased the liver phosphoprotein phosphorus (PPP) significantly, confirming the observations of Chapman *et al.* (1). There were not any significant effects from the superimposition of the aminopterin treatments on the hormonal treatments.

Experiment II

A second experiment was carried out in the light of the foregoing results and with the object of studying the ability of certain treatments to overcome the various effects of aminopterin in the responses to hormonal treatments.

On the basis of the results of Experiment I, it was decided that 0.25 mgm. aminopterin per diem would be appropriate and would avoid depression of appetite.

The experimental technique, type of bird, and analytical methods were the same as in Experiment I.

The additional treatments selected for study were firstly, intraperitoneal injection of appropriate amounts of folic acid (Nutritional Biochemicals), and secondly, of sodium desoxyribonucleate (Nutritional Biochemicals). Neither treatment affected appetite adversely. If aminopterin operates by depressing desoxyribonucleoside synthesis and hence desoxyribonucleic acid synthesis, then the effects of aminopterin might be overcome by supplying desoxyribonucleic acid, and it was for this reason that the effects of desoxyribonucleic acid were examined. The results are set out in Table II.

Aminopterin depressed the response of the oviduct to estrogen (comparison of Groups A and B), and folic acid antagonized this effect of aminopterin (comparison of Groups A, B, and C). The data for Group D show that the treatment with desoxyribonucleic acid did not affect the response of the oviduct significantly in this experiment. The inhibition of oviduct response, however, was rather small. It is evident that a slightly higher level of aminopterin would have been preferable for the purposes of this experiment.

Aminopterin did not significantly affect the serum calcium level (comparison of Groups A and B), a result in general agreement with the observations in Experiment I. The serum calcium levels of Groups A and D were the same. However, the serum calcium level in Group C was significantly lower than those of the other groups. In other words, the folic acid treatment superimposed on the aminopterin treatment gave a net reduction of serum calcium

TABLE II

EFFECTS OF TREATMENT WITH FOLIC ACID OR DESOXYRIBONUCLEIC ACID ON THE MODIFICATION OF CERTAIN RESPONSES OF THE IMMATURE PULLET TO ESTROGEN PLUS ANDROGEN. DURATION OF EXPERIMENT, 12 DAYS ALL BIRDS RECEIVED 1 MGM. ESTRADIOL BENZOATE PLUS 0.5 MGM. TESTOSTERONE PROPIONATE DAILY. AVERAGE DATA FOR SIX BIRDS ON EACH TREATMENT

Group	A	B	C	D	
Aminopterin mgm./day Supplementary treatment	Nil Nil	0.25 Nil	0.25 600 mgm. folic acid	0.25 3 gm. DNA	L.S.D. $P = 0.05$
Initial live weight, kgm. Final live weight, kgm. Gain, kgm.	1.14 1.36 0.22	1.16 1.32 0.16	1.30 1.39 0.09	1.21 1.33 0.12	0.11
Feed consumption, kgm. Serum Ca, mgm./100 ml.	0.84 70	0.84 79.3	0.84 52	0.84 70	12.9
Oviduct, gm.	24	15	22	17	4.9
Spleen, gm.*	1.81	1.49	2.56	1.94	0.79 (0.66 at $P = 0.1$)
Adrenals, gm.	151	113	119	109	23
DNAP mgm./100 gm. liver DNAPmgm./kgm. live weight	26.3 7.67	25.2 7.33	24.4 7.36	27.5 7.41	N.S.
RNAP mgm./100 gm. liver RNAPmgm./kgm. live weight	75.4 22.0	77.4 22.5	74.9 22.5	74.6 20.3	N.S.
Ratio $\frac{\text{RNAP}}{\text{DNAP}}$	2.9	3.1	3.1	2.8	N.S.

* *By the "t" test on individual pairs of groups*
Gr. A vs. Gr. B Not significant.
Gr. B vs. Gr. D Highly significant.

level. It is hoped further to investigate this result, which suggests that a certain degree of folic acid deficiency may enhance the effect of estrogen on serum calcium level.

The spleen weight of Group B was less than the spleen weight of Group A, but the effect did not attain significance either by the "*F*" test or the "*t*" test. The spleen weight of Group C was significantly greater than the spleen weights of the other three groups. The folic acid treatment, therefore, not merely antagonized the possible effect of aminopterin, but enhanced the spleen weight above that of the control Group. Villela and Mello (15) have recorded that folic acid increased the size and weight of the spleen in rats. This response of spleen weight may tentatively be associated with a positive effect of folic acid on the hematopoietic system.

The results for the spleen weights of Group D are specially interesting, for the spleen weight of this Group was significantly greater than that of Group B

by the "*t*" test, though not significantly greater than that of Group A. The most reasonable explanation of the observations is that the treatment with desoxyribonucleic acid antagonized a depressant effect of aminopterin on spleen weight. This observation constituted the only reasonably definite effect of desoxyribonucleic acid observed in the present experiments.

It has been observed in other unpublished work in this laboratory that treatment with estrogen will significantly increase adrenal weight in the immature pullet. Timiras (14) has observed a similar effect in rats, whose adrenal weight was increased by injections of stilbestrol. It is legitimate, therefore, to regard the adrenal weight of Group A as greater than it would have been in nonestrogenized pullets. On this basis the tendency for lower adrenal weight in Group B might seem to be real, though not significant, and the higher value of Group D would appear to be an effect of desoxyribonucleic acid in antagonizing the effects of aminopterin.

The changes in liver nucleic acids were slight and none of the differences attained significance. If any effects exist, then it would appear that greater dosage levels of aminopterin and of desoxyribonucleic acid would be necessary before any effects on liver nucleic acids would be evident. This suggestion is in accord with the obvious absence of any effect of the lower levels of aminopterin on liver DNAP in Experiment I.

Acknowledgments

The authors wish to express their thanks to the National Research Council of Canada for the grant which made this work possible. They are happy once more to express their gratitude to Messrs. Schering Limited, Montreal, for generous gifts of hormones. The aminopterin was presented by Dr. T. H. Jukes of the Lederle Laboratories, and the authors take this opportunity of thanking him, as well as those colleagues who cheerfully gave of their time in helping at certain rush periods of the work.

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A STUDY OF THE LIFE HISTORY OF *TRICHOBILHARZIA CAMERONI* SP. NOV. (FAMILY SCHISTOSOMATIDAE)[1]

By Liang-Yu Wu[2]

Abstract

A cause of swimmer's itch in the lower Ottawa River is *Trichobilharzia cameroni* sp. nov. Its life cycle has been completed experimentally in laboratory-bred snails and in canaries and ducks, and the various stages are described. The eggs are spindle-shaped. The sporocysts are colorless and tubular. Mother sporocysts become mature in about a week. The younger daughter sporocyst is provided with spines on the anterior end and becomes mature in about three weeks. The development in the snail requires from 28 to 35 days. A few cercariae were found to live for up to 14 days at 50° C., although their life at 16° to 18° C. was about four days. Cercariae kept at room temperature for 60 to 72 hr. were found infective. The adults become mature in canaries and pass eggs in about 12 to 14 days. *Physa gyrina* is the species of snail naturally infected. It was found in one case giving off cercariae for five months after being kept in the laboratory. Domestic ducks were found to become infected until they were at least four months old, with the parasites developing to maturity in due course; no experiments were made with older ducks. Furthermore, miracidia were still recovered from the faeces four months after the duck had been experimentally infected, and it is suggested that migratory birds are the source of the local infection.

Introduction

An outbreak of swimmer's itch in the vicinity of Ste. Anne de Bellevue near Montreal, Que., during the summer of 1949, initiated investigations on this problem by members of the Institute of Parasitology. The dermatitis schistosome seems to be present sporadically along the Ottawa River in the vicinity of this town, because cases are frequently reported. A more thorough study of the same problem was made during the late fall and winter of 1951. The purpose of this study was to elucidate the biology of the parasites concerned in the hope that our understanding of them may suggest some means for their control. The local species was identified as belonging to the genus *Trichobilharzia*.

Historical Review

The genus *Trichobilharzia* belongs to the subfamily Bilharziellinae of the family Schistosomatidae, and was created by Skrjabin and Zakharow, 1920, for their species *T. kossarowi* from wild ducks in Russia. Brumpt (1), by experimental infection of domestic ducks with *Cercaria ocellata* from *Limnaea stagnalis*, subsequently recovered eggs and fragments of the adults from those ducks. He identified them as *T. kossarowi;* because of priority, he proposed that the name should become *Trichobilharzia ocellata* (La Vallete, 1854).

Oiso (10) described *Bilharziella yokogawai* from an experimental infection of ducks in Taiwan (Formosa). Because of the long and narrower posterior portion of its body (instead of a narrow anterior and a broader lanceolate

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posterior end), it was placed in the genus *Pseudobilharziella* by McLeod (5). Ejsmont (3) had established this genus for the elongated filamentous male specimens described by Kowalewski in 1859 as young males of *Bilharzia polonica* in Russia. *Pseudobilharziella* was created chiefly on the basis of the presence of the gynecophoral fold; Kowalewski's misidentified material was renamed *P. kowalewskii.*

McLeod (5) described *Pseudobilharziella querquedullae* from *Querquedula discors*, based on male specimens only. A few years later, he infected birds experimentally with *Cercaria physellae* Talbot, and completed the description for the female (6). The eggs of McLeod's species are very similar to those of *Bilharziella yokogawai*, the chief difference between the adults lying in the strikingly forward position of the gynecophoral fold in the latter. *P. querquedullae* was later considered a synonym of *Trichobilharzia physellae* by McMullen and Beaver (7).

Szidat (12) described *Pseudobilharziella filiformis* from *Cygnus olor.* The eggs are oval and carry a small spine at the broader end.

Yamaguti (16) described a new species, *Pseudobilharziella corvi* from *Corvus corone corone* from Japan.

McMullen and Beaver (7) by experimental infection of birds with *Cercaria physellae* Talbot, 1936, *Cercaria stagnicolae* Talbot, 1936, and *Cercaria elvae* Miller, 1923, recovered the adults and eggs of three species of trematodes which they identified as *Trichobilharzia physellae* (Talbot), *T. stagnicolae* (Talbot), and *T. ocellata. T. ocellata* from *Cercaria elvae* was considered by these authors to be identical to that described by Brumpt (1) because of the similarity of the eggs produced.

They raised the question of the identity of the genus *Pseudobilharziella.* According to their opinion:

> "Ejsmont (1929) established the genus *Pseudobilharziella* largely on the basis of the presence of a gynecophoric canal, but a study of the genera in question indicates that this structure is present in both. Consequently *Pseudobilharziella* Ejsmont, 1929, becomes a synonym of *Trichobilharzia* Skrjabin and Zakharow, 1920."

Consequently, they proposed to transfer all the species so far described under the genus *Pseudobilharziella* to the genus *Trichobilharzia.*

Materials and Methods

Snails of the species *Physa gyrina* were collected from a beach of the Ottawa River from which swimmer's itch had been reported during the summer of 1951. Eleven of these snails were found giving off the same species of schistosome cercariae previously found responsible for swimmer's itch. These cercariae were used for experimental infections but during the latter part of the study, cercariae from experimentally infected snails were used. The snails used for the study of the various stages of the life history were laboratory-bred.

The birds used for experimental infections were canaries, ducks, and pigeons. The young canaries and pigeons, one or two weeks old, were obtained from breeders. The ducklings, about two weeks old, came from Brome Lake Farm. All were kept in the laboratory throughout the study.

The eggs of the parasites were studied in a fresh condition in saline and water. They were preserved in hot Gilson's fluid which causes less shrinkage.

The droppings of the infected canaries were placed in serological test tubes with well water, the miracidia which hatched being removed for study and experimental infections. The miracidia were studied unstained or stained with vital stains, for example, neutral red, methyl blue, Nile blue, brilliant cresyl blue, and Janus green. Methyl cellulose was also used to reduce the activity of the larvae. They were measured after fixation in Bouin's fixative and Gilson's fluid.

The sporocysts were studied fresh as well as fixed with Gilson's fluid and stained with borax carmine.

The cercariae from naturally infected snails were studied alive, unstained or stained with vital stains. They were stained with very dilute neutral red and fixed in 10% hot formalin (15) for measurement.

The adults were obtained from the experimentally infected birds. The birds were injected with 1% sodium citrate through the heart; soon after they were killed, to prevent blood coagulation. The parasites remained alive for five or six hours or even longer after the birds were dead. The number of parasites recovered from each bird was small. The parasites were in the peripheral blood vessels on the intestinal wall. When a worm was located, a break was made on the vessel about the middle of the worm which could then be squeezed out slowly by pressing at both ends. The adults were also frequently found in the liver which was teased in saline as soon as the birds were killed. After a couple of hours many adults were found in the saline or were partly exposed and could be dissected out with ease. The morphology of the adults was studied in unfixed specimens as well as in specimens fixed in Gilson's fluid and stained with borax carmine and Harrison's haematoxylin. They were fixed in Gilson's fluid and Bouin's fixative for sectioning.

The cercariae from the naturally infected snails were used for experimental infections. Approximately equal numbers of cercariae from each snail were used in order to ensure that both males and females were present. The birds to be infected were held in such a position that their feet were suspended in the vessel containing the cercariae. The time of exposure was usually from one and a half hours to two hours. The infection was judged by the approximate number of cercariae left in the vessel.

Single snails were infected by exposing each to three to six miracidia for a few hours.

Life History

Intermediate Hosts

A high percentage of laboratory-bred *P. gyrina* developed infections following exposure to miracidia. The latter disappeared within half an hour after they were placed with the snails. These snails were small and lived for a few weeks only after the cercariae began to emerge, whereas one specimen, infected naturally, lived for five and a half months. Cercariae emerged daily from it; as many as 138 emerged in one night four and a half months after its capture, and 43 in one night some three weeks later. Attempts to infect *S. palustris* and *L. stagnalis* were unsuccessful.

Experimental Definitive Hosts

Canaries are very convenient for obtaining material for study, but are quite sensitive to the parasites and the infections are low. A heavy infection usually resulted in pneumonic symptoms when the young parasites passed through the lungs and caused the death of the birds. Fourteen canaries were used in the experimental infections.

On post-mortem examination young flukes were found in the lungs, kidneys, livers, and mesenteric blood vessels. Some obtained as early as 24 hr. after infection had the disintegrated remains of the eyespots scattered as dark brown granules between the oral and ventral suckers. In older infections eggs were found in the veins of the wall of the small intestine and the liver, and eggs were passed in the droppings of one bird, a fortnight after infection. Adults were recovered from the liver and from the rectum on post-mortem examination, although males and females were never recovered together.

Nine ducklings, between two and three weeks old, were exposed to infection and most developed moderate infections, eggs being found in the walls of both small and large intestines; portions of intestine containing these eggs were kept at 3°–4° C. for two weeks and miracidia were subsequently recovered from them. Young flukes were found in the lungs of one bird four days after infection and in older infections mature flukes were recovered from the veins of the caeca and large intestine.

Four older ducks, up to four months old, were infected but infections were light. The young ducklings seemed to be more susceptible but, although the number of cercariae used for infection was large, between 500 and 5000, only a few schistosomes reached maturity.

Four pigeons were exposed to infection which in two cases was successful. Both were young birds under two months old.

Adults

The adults are long, slender, and delicate worms. The body is slightly flattened at the anterior end and appears to be oval in cross section. In some specimens they are greatly flattened in front of the gynecophoral fold in the males and in the corresponding region in the females. The suckers are fairly well developed. The oral sucker is terminal. Its dorsal wall is thicker than

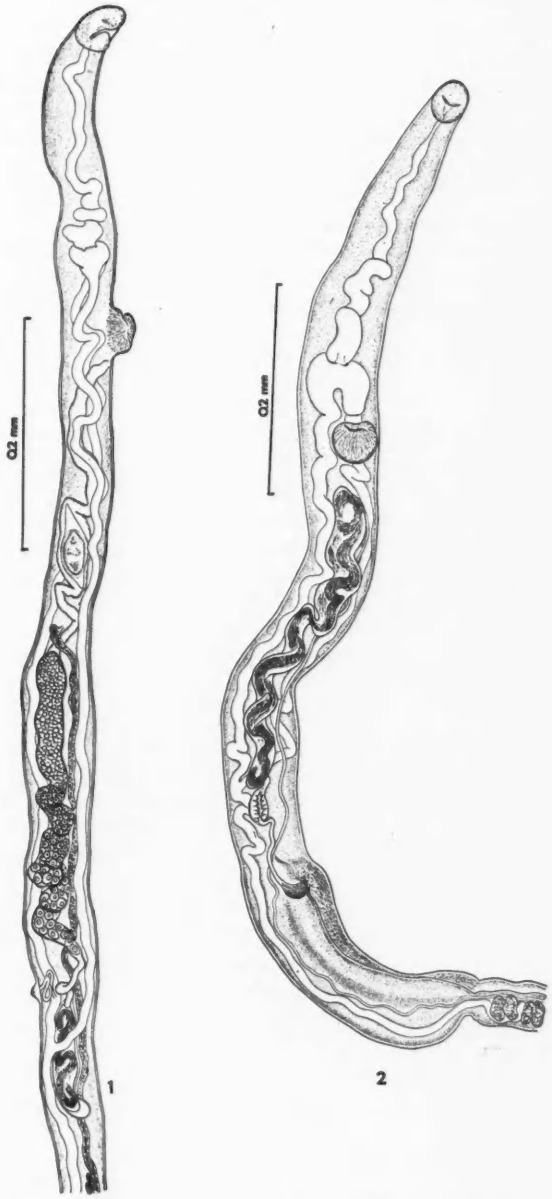


FIG. 1. Anterior end of a female *Trichobilharzia cameroni* sp. nov.
FIG. 2. Anterior end of a male *Trichobilharzia cameroni* sp. nov.

the ventral wall and the opening is subterminal. The ventral sucker is solid and thickly set with spines. It has never been seen retracted. The cuticle is apparently smooth and no spines are definitely seen. The fine striations are often present on the anterior end. In some females the cuticle is somewhat tuberculated. The posterior end in both sexes is flattened and expanded into a somewhat trilobed spatulate structure. The oesophagus is simple and very long. It is greatly enlarged a short distance before the acetabulum after which it bifurcates. These branches reunite at about the same level in both sexes, at the posterior end of the reproductive glands. The caecum passes through the rest of the body in a zigzag course in the semicontracted specimen, and ends a short distance from or very close to the posterior extremity. The excretory pore is terminal. The excretory bladder is a very small, subspherical sac lying close to the posterior extremity and discharges to the exterior through a shot canal.

Male

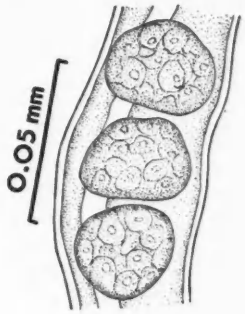
Measurements of 23 moderately to well extended fixed specimens (Fig. 2) from canaries, pigeons, and ducks vary from 3.18 to 5.71 mm. long, the longest being from the duck. The greatest width is at the gynecophoral fold which cannot be measured as the margins are always inrolled on themselves. It is a well developed fold and thickly set with spines on the ventral surface (Fig. 5). The long axis of the fold is usually arched. The measurement along the median line varies from 0.212 to 0.345 mm. When it is contracted, it is much shorter and broader (Fig. 7). The width just behind the acetabulum (measured from 12 specimens) varies from 0.063 to 0.097 mm.

The oral suckers from eight specimens vary from 0.031 to 0.040 mm. in width by 0.038 to 0.047 mm. in length. The ventral sucker is greater in cross diameter. The measurement of the ventral suckers, shown more or less in lateral view from seven specimens, varies from 0.054 to 0.064 mm., and the measurement of five ventral suckers shown in front view varies from 0.031 to 0.049 mm. by 0.045 to 0.051 mm. The ventral sucker is usually closer to the oral sucker than to the gynecophoral fold, although in some specimens it is about midway between them.

The intestinal bifurcation is at a short distance in front of the ventral sucker, and the reunion is about the level of the prostate gland. In one moderately extended living specimen, in which the digestive tract was full of red blood cells flowing back and forth, the union was seen just at the posterior margin of the prostate gland. The caecum passes backward, looping between the testes in contracted specimens and lying straight on one side of the body in well extended specimens.

The testes are numerous. In nine specimens examined the number varied from about 80 to 110. They are arranged in a single row (Fig. 3), beginning immediately behind the gynecophoral fold and ending a short distance before the tip of the intestinal caecum (Fig. 8). They are tandem in well extended specimens and irregularly alternated on either side of the intestinal caecum in the contracted specimens (Figs. 4 and 6). Their shapes are spherical,

subspherical, or oval. Random measurements of some testes from the extended specimens vary from 0.020 to 0.026 mm. in diameter and from 0.017 to 0.031 mm. by 0.020 to 0.041 mm. Those near the posterior extremity are much smaller. The vas deferens passes forward from the posterior end and discharges into the anterior end of the seminal vesicle. The seminal vesicle is a convoluted tube which is divided into two portions by a constriction, the anterior portion staining more deeply. It extends from a short



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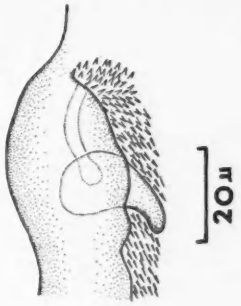

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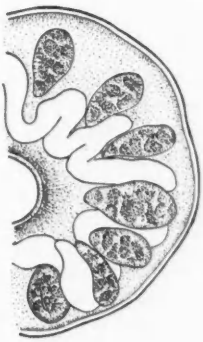

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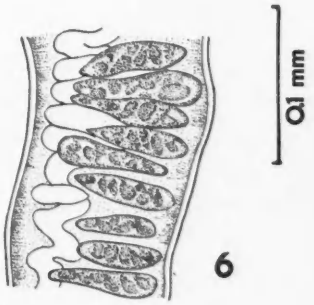

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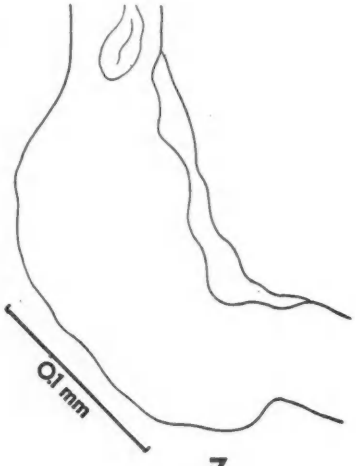
FIG. 3. A portion of the posterior end of an extended male, showing the normal shapes and the linear arrangement of the testes.

FIG. 4. A portion of a male, bending, showing the intestinal caecum and the club-shaped testes due to pressure.

FIG. 5. The anterior right portion of the gynecophoral fold showing the spines and the genital papilla.

FIG. 6. A contracted portion of a male, showing the caecum and the elongated testes due to contraction.



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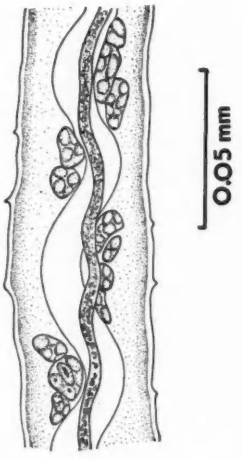

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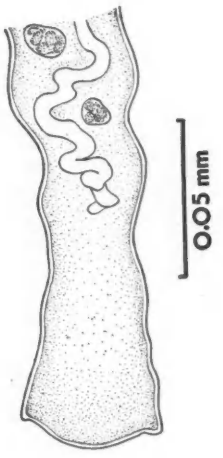

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FIG. 7. A contracted gynecophoral fold of a male.

FIG. 8. Posterior end of male showing two testes and the caecum.

FIG. 9. A portion of a female showing the vitelline duct and groups of vitellaria alternating with the caecum.

distance behind the ventral sucker to a short distance in front of the gynecophoral fold. In some specimens its anterior border is just behind the acetabulum, depending on the contraction of the body. It opens into a well developed prostate gland, which in turn, passes by a narrow ejaculatory duct into a saclike cirrus. The cirrus opening is on a genital papilla on the right margin of the gynecophoral fold (Fig. 5). The prostate gland and the posterior end of the seminal vesicle are enclosed in a sac.

The distance between the oral sucker and the acetabulum varies from 0.226 to 0.370 mm., and the distance between the acetabulum and the gynecophoral fold varies from 0.230 to 0.536 mm.

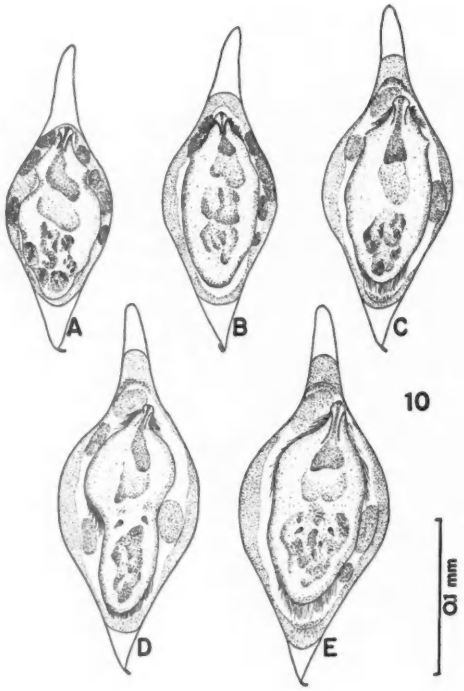
Female

The females (Fig. 1) are somewhat shorter and narrower. Measurements of six moderately to well extended specimens from canaries varied from 3.82 to 4.94 mm. The greatest width, at the region of the ovary and the seminal receptacle, is from 0.052 to 0.059 mm.

The oral suckers from seven specimens, measured from 0.025 to 0.034 mm. wide and 0.032 to 0.040 mm. long. The acetabula measured from 13 specimens were from 0.030 to 0.047 mm. wide and 0.025 to 0.038 mm. long.

The distance between the anterior end and the acetabulum is from 0.213 to 0.342 mm. and the distance between the acetabulum and the ovary is from 0.179 to 0.250 mm. The intestinal bifurcation is at a short distance in front of the ventral sucker. The intestinal reunion was seen about the equator of the seminal receptable and the caecum passes through the long posterior end of the body in a zigzag course and ends near the posterior extremity, as in the male.

The ovary is tubular, stout and spiral, making several loops. Its length in seven specimens varied from 0.016 to 0.033 mm. by 0.287 to 0.358 mm. It becomes narrower at the posterior end where it leads into the oviduct. The oviduct is a moderately long stout duct, which expands into a convoluted, saclike seminal receptacle. The length of the seminal receptacle (from five specimens) varied from 0.018 to 0.042 mm. by 0.072 to 0.125 mm. It leads, at its posterior end, into the uterus which bends sharply forward and opens through the genital opening immediately behind the acetabulum. The female genital opening was only seen on one occasion; it appears to have a thick rim. The uterus is a convoluted tube. The oötype was not observed. There is never more than a single uterine egg. Laurer's canal is a small coiled duct, discharging through an opening on a prominent papilla on the dorsal surface of the body in front of the seminal receptacle. The vitellaria extend from just behind the seminal receptacle to the posterior end of the body, a short distance in front of the tip of the caecum. They are usually in groups of two or three, arranged irregularly, alternating with the intestinal loops (Fig. 9). The single vitelline duct, a fairly large duct, passes forward ventral to the seminal receptacle and the ovary, and it appears to join the uterus just in front of the ovary.



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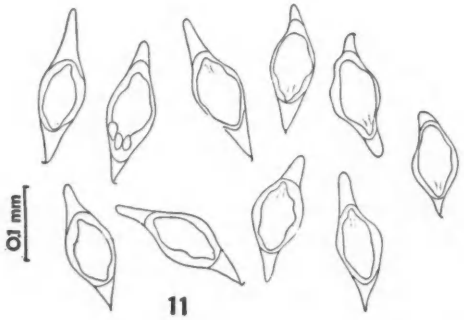

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FIG. 10*a*. A normal egg in saline.
10*b*. The same egg, 20 min. after saline was replaced with water.
10*c*. In water 30 min.
10*d*. In water 37 min.
10*e*. In water 43 min.

FIG. 11. Ten random eggs showing variations in shape.

Egg

The egg is spindle-shaped (Fig. 10), with a thin colorless shell. The axis is usually straight but sometimes may be slightly curved or otherwise varied (Fig. 11). It tapers gradually at both ends giving rise to a fairly long pole ending in a blunt tip, and a shorter, conical opposite pole carrying a short projection which is usually bent like a hook. Measurements of 35 eggs from canaries with living miracidia inside, examined in saline, varied from 0.147 by 0.057 mm. to 0.212 by 0.063 mm., the greatest length, and 0.181 mm. by 0.073 mm., the greatest width. Measurement of another 35 eggs in water varied from 0.066 by 0.198 mm. to 0.080 by 0.24 mm., the greatest width, and 0.188 by 0.070 mm. to 0.253 mm. by 0.077 mm., the greatest length. The larger size of the egg in water is due to the absorption of water (Fig. 10). The anterior end of the miracidium may be toward either pole of the egg. Between the miracidium and the shell, there is a layer of thick, semisolid, greenish material, as well as vestigial protein and excretory granules.

Hatching usually takes place in from two to five hours, after the eggs are put into water, although some take as long as eight hours. Under the microscope, the entire process of hatching has been observed. An egg from the droppings of a canary was removed in saline and was studied in saline under a cover glass. The egg measured 70 by 191μ (Fig. 10*a*). The miracidium was usually motionless in saline although it occasionally showed laborious movement, usually limited to the anterior end. Then the saline was replaced by water. After 20 min. the egg measured 77 by 209μ (Fig. 10*b*). A narrow space appeared between the miracidium and the semifluid substance. The miracidium began to show constant movement, but no ciliary vibrations were seen. After half an hour it measured 90 by 226μ (Fig. 10*c*). The space between the miracidium and the shell gradually increased, indicating that more water was absorbed. Then ciliary action began. In 37 min. it measured 94 by 233μ and the miracidium was moving actively (Fig. 10*d*). In 43 min., the size of the egg was greatly increased and the miracidium was moving vigorously. Before the size could be determined, the egg shell ruptured and the miracidium escaped. The splitting of the egg shell always occurred about the middle third of the egg.

Miracidium

The miracidium (Fig. 12) appears to be cylindrical with two slight constrictions. It rotates with a spiral movement as it moves rapidly in the water. The anterior end tapers abruptly to a conical papilla. The body has numerous long cilia, in patches arranged in four transverse rows. This arrangement of cilia appears to correspond to the arrangement of the epidermal cells as observed in other schistosome miracidia. The shape and size of the epidermal cells were not studied in detail.

The measurement of 12 less contracted specimens fixed in Bouin's fixative varied from 38 to 44μ by 110 to 146μ, and 12 less contracted specimens in

Gilson's fluid, from 38 to 45μ by 112 to 129μ. The size of a few living specimens, stained with very dilute neutral red or in methyl cellulose and under a cover glass, is from 175 to 190μ by 35 to 42μ.

There are two pairs of flame cells, one located lateral to the penetration glands and one about one-third the body length from the posterior end. The flame cells on each side are connected with two long collecting tubules. The anterior collecting tubule after making a mass of loose loops just behind the

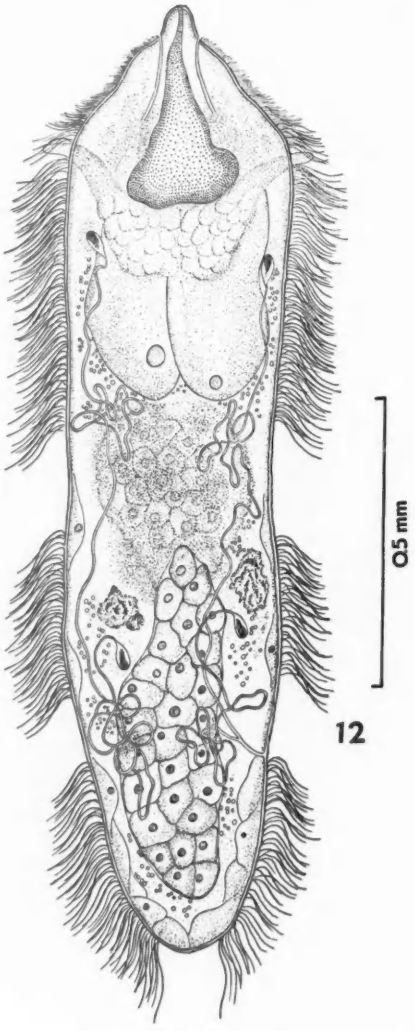

FIG. 12. A miracidium.

penetration gland passes backward and unites with the posterior collecting tubule, which also makes a mass of loops, into a short common collecting tube which opens laterally to the exterior at about one-fourth the body length from the posterior end.

The "primitive gut" is a flask-shaped gland. It is coarsely granulated and stains with basic dyes. It opens at the tip of the anterior papilla. There are two large penetration glands which occupy about the anterior two-fifths of the body, and open by ducts on each side of the base of the anterior papilla. They are homogeneous in appearance and each has a single nucleus. They are not stained with either basic or acid dyes.

The lateral secretory glands consist of two clusters of unicellular glands, similar to those of the human schistosomes (Faust (4)). Their openings were not determined but must be associated with the two lateral processes. They also do not take stains. The lateral processes are the two prominent lateral tubular processes one on each side of the body just behind the first row of cilia. In this species, the penetration glands are found posterior to the lateral secretory glands, a condition differing from that shown by Brumpt (1) for *T. ocellata*.

The germ cells occupy the major portion of the posterior half of the body. They are more or less square and closely fitted together. They appear to be contained in an elongated, elastic sac, because it retains its saclike structure, even when its shape changes during contraction and expansion of the miracidium. Each germ cell contains a large nucleus. The arrangement of the germ cells in this species differs from that in *Schistosomatium douthitti* (11). According to her description, the germ cells are attached to the body wall by fiberlike extensions of the body.

Most of about 125 miracidia observed lived from 2 to 13 hr. but a few remained alive up to 17 to 21 hr., but were inactive and near the bottom of the test tube. The penetration power of the miracidium appears to be limited to a rather short time and apparently gradually decreases two or three hours after hatching.

First Generation Sporocyst

The mother sporocysts (Fig. 13) are tubular and very slender, much longer than the daughter sporocysts. They are colorless and transparent. Two mature sporocysts with migrating daughter sporocysts, from the liver of a *Physa*, 12 days after infection, measured 3.84 by 0.12 mm. and 4.25 by 0.125 mm., respectively. The birth pore was not seen. The lumen contains motile daughter sporocysts, germ cells, and germ balls in different stages of development.

The mother sporocysts were found in the liver, the margin of the mantle, and the margin of the foot. Sometimes they were found attached to the viscera. The mother sporocysts may become mature in as little time as a week, as a small number of daughter sporocysts were found in a snail eight days after infection.

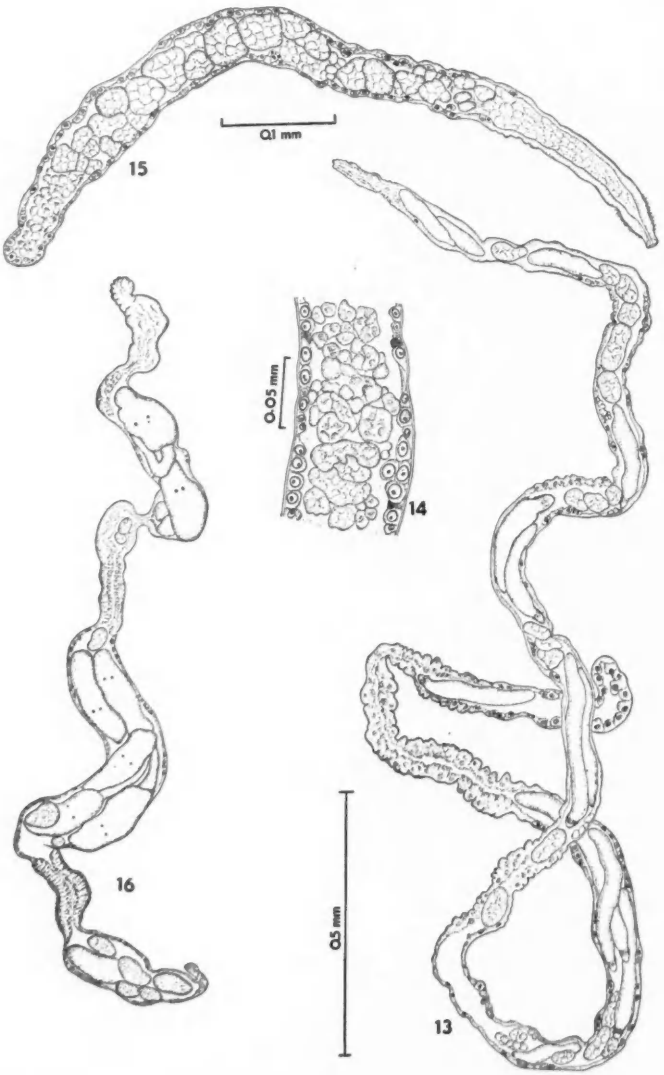

FIG. 13. A portion of a mother sporocyst, nine days after infection, showing the germ balls and germ cells embedded in the wall of the sporocyst.

FIG. 14. A mature sporocyst, from an experimentally infected snail with migrating second daughter sporocyst and germ balls.

FIG. 15. A young daughter sporocyst from an experimentally infected snail, showing germ cells, germ masses, and spines at the anterior end.

FIG. 16. A mature daughter sporocyst from an experimentally infected snail, showing germ masses and the migrating cercaria.

The spines at the anterior end of the daughter sporocysts are visible. These migrating daughter sporocysts with the spines serve as a means of differentiating the mother sporocysts from the daughter sporocysts that contain cercarial embryos with tails not yet developed.

Second Generation Sporocyst

The daughter sporocysts have the same general appearance as the mother sporocysts but are much shorter. The young daughter sporocysts (Fig. 15) bear many stiff spines near the anterior end. The birth pore is terminal at the anterior extremity and communicates with the body cavity by a short narrow birth canal. The body wall is similar to that found in the mother sporocyst. The lumen is fully packed with germinal material, except at the very anterior portion. The young sporocysts are reasonably active, showing active contraction and expansion, especially at the anterior end, with the germinal materials in the lumen flowing back and forth. The spines are only present in the young forms, disappearing later. Some of the germ cells on the body wall were seen projecting into the lumen. These may be set free later, because many single germ cells were found in the lumen. In a young sporocyst, 12 days after infection, the germinal materials consist of germinal masses in different stages of development, ranging from single cells to masses containing many cells; in one sporocyst three days older, some of the embryos were much larger and had become elongated, the cellular components being no longer visible.

The mature sporocysts (Fig. 16) are also active, showing contraction and extension. The germ cells in the protoplasmic layer are not so numerous as in the younger forms. In addition to a small number of germ cells and germ balls, there are cercariae both motile and nonmotile. The measurements of 15 mature sporocysts, 35 days after infection and containing motile cercariae, varied from 0.07 to 0.15 mm. by 0.98 to 2.99 mm. The width varies with the number of cercariae accumulated, as the wall is very elastic. The number of motile cercariae with eyespots was usually from two to five, but in one case 10 motile cercariae were seen.

The daughter sporocysts develop in the liver of the snail. They are usually numerous and closely packed. They are also closely associated with the host tissue, making it difficult to separate them. It takes 28 to 35 days for the cercariae to emerge after the snails were exposed to the miracidia. Therefore it takes about three weeks for the daughter sporocysts to become mature and produce the cercariae.

Cercaria

The cercariae (Fig. 17) are apharyngeal and ferocercous with pigmented eyespots. Since the morphology of the cercariae is very similar in different related bird schistosomes, many workers consider the measurement and proportions of importance. This species was measured in both ventral and lateral views (see Table I).

TABLE I

MEASUREMENT OF 30 CERCARIAE OF *T. cameroni*, MM.

	Ventral view				Lateral view			
	Maximum	Minimum	Mean	Standard deviation	Maximum	Minimum	Mean	Standard deviation
Length of body	0.361	0.257	0.319	± 0.0256	0.347	0.296	0.329	± 0.0131
Width of body	0.070	0.052	0.057	± 0.0040	0.059	0.049	0.054	± 0.0026
Length of head organ	0.132	0.080	0.108	± 0.0113	0.125	0.090	0.110	± 0.0119
Width of head organ	0.046	0.028	0.038	± 0.0038	0.044	0.035	0.038	± 0.0018
Length of ventral sucker	0.031	0.024	0.030	± 0.0020	0.035	0.031	0.032	± 0.0011
Width of ventral sucker	0.035	0.028	0.032	± 0.0024	—	—	—	—
Distance from anterior end to ventral sucker	0.219	0.139	0.187	± 0.0215	0.237	0.184	0.209	± 0.0111
Distance from posterior end to ventral sucker	0.111	0.073	0.090	± 0.0119	0.104	0.077	0.086	± 0.0062
Length of tail stem	0.424	0.347	0.369	± 0.0242	0.382	0.344	0.363	± 0.0106
Width of tail stem	0.042	0.035	0.038	± 0.0015	0.040	0.031	0.037	± 0.0022
Length of tail furca	0.253	0.195	0.225	± 0.0114	0.240	0.195	0.220	± 0.0092
Width of tail furca	0.026	0.019	0.023	± 0.0014	0.038	0.029	0.033	± 0.0070

The measurement of the lateral view (taken along the dotted lines indicated in the figure (Fig. 19)) shows less variation. This is because the cercariae attach themselves to a surface with the ventral suckers and with the anterior and posterior ends bent away, and after fixation, many of them lie on their sides in a relaxed condition.

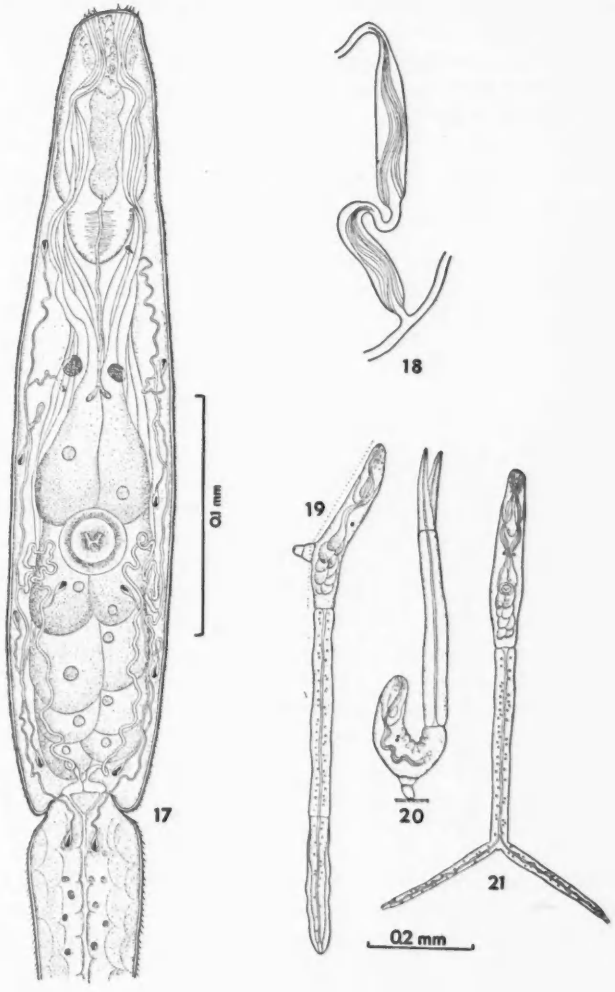

FIG. 17. The body of a cercaria with the basal portion of its tail.
FIG. 18. An enlarged sketch of the ciliary patches.
FIG. 19. Lateral view of a formalin preserved cercaria.
FIG. 20. A living cercaria attached to the substratum with its ventral sucker and with the preacetabular region and tail bending away.
FIG. 21. Ventral view of a fairly well extended formalin preserved cercaria.

The entire surface is covered with spines. Those on the body are very fine and those on the tail are coarser and longer. The head organ is a muscular one, particularly the posterior end. The ventral sucker is subspherical. It is a very muscular and telescope-like structure, projecting and retracting, and, like the body surface, is provided with spines.

There are five pairs of penetration glands. Two pairs of circumacetabular glands are more granular. The three postacetabular glands are more homogeneous. The ducts of these glands pass forward dorsally and, bending ventrally near the head organ, lie ventral to it. They open to the exterior in two groups of five papillae each. Each papilla is provided with a sharp spine.

The digestive system consists of a slender, narrow oesophagus which divides a short distance posterior to the eyespots into two small saclike caeca.

The excretory system consists of seven pairs of flame cells, six pairs in the body and one pair in the base of the tail, $2\,[\,(3) + (3 + 1)\,]$. There are one pair of anterior and one pair of posterior collecting tubes connected with each flame cell by a fine capillary tubule. The two collecting tubes on the same side of the body unite at a short distance behind and lateral to the ventral sucker into a common collecting tube which immediately gives rise to an enlarged loop containing two ciliary patches (Fig. 18). Each common collecting tube passes backward and inward, lying ventral to the penetration glands. Each opens into a small bladder, lying at the posterior extremity of the body. In turn, each bladder discharges into a small tube which passes into the base of the tail, around the 'island of Cort', and unites with the corresponding tube from the other side into a single common excretory tube. This common excretory tube passes through the stem of the tail and bifurcates near the posterior end of the stem. Each branch passes into a furca and eventually discharges through the tip of the furca. The anterior loop of the common collecting tube usually reaches the equator of the ventral sucker; sometimes it also reaches the anterior border or the posterior border of the ventral sucker depending on the contraction of the body. The two small excretory bladders are close together. There is a partition between them which can easily be seen in a specimen under a cover glass and with slight pressure. This condition differs from the single bladder described by other authors.

The furcae are provided with dorsoventral fin folds which extend from near the base of the furcae to the tips of the furcae. The width of the furcae measured from the lateral view represents their actual width. Each furca contains from 15 to 22 nuclei.

The cercariae are positively phototactic. They are usually found attached to the wall of the container near the water surface on the side facing the light. If the container is disturbed and turned round, they swim actively across the container toward the lighted side and again attach. Occasionally they are seen suspended in the water with their ventral suckers attached to the surface film. The tail and the anterior end bend away in the usual manner. Sometimes, especially after they have been in the water for a few days, they are

found on the bottom of the container. In a shallow dish and under the binocular microscope they are seen attached by the powerful ventral suckers. The anterior end and the postacetabular region together with the tail, bend backward, parallel to each other and perpendicular to the surface of attachment (Fig. 20). The furcae relax making various angles, less than 45 degrees, approaching or crossing each other. In this respect, this species is similar to that of some of the Ocellata group in Europe (Sinitzin, 1910; (14)). Under a cover glass the body and the tail rotate with the ventral sucker as a fulcrum. The cercariae may move on the slide with the head organ and the ventral sucker attaching and releasing alternately together with the contraction and extension of the preacetabular portion of the body.

Several series of observations on the longevity of the cercariae were made. Most died after three days at room temperature. At a temperature of 16° to 18° C., they lived much longer, the majority living up to four days, remaining active, and attached to the side of the container. A few lived up to six days, but all were sluggish by then. The cercariae lived longest at a temperature of about 5° C., but owing to the cold most of them were found on the bottom of the container, moving rather sluggishly. Many of them lived up to four or five days but a small percentage remained alive for from 12 days to two weeks.

Discussion

This species is very similar to both *Trichobilharzia physellae* as described by McMullen and Beaver (8) and *Pseudobilharziella querquedullae* as described by McLeod (5, 6). McMullen and Beaver regard these two species as identical but there is a number of minor differences between them, which makes it seem desirable to retain them as separate species pending the examination of further material. There seems little doubt, however, that they are congeneric and McLeod's species is, accordingly, placed in the genus *Trichobilharzia* as *T. querquedullae* (McLeod). The differences between the material under study and the two previously described forms are sufficient to describe it as new with the name *Trichobilharzia cameroni*.

Adults

The males are only slightly longer than the females of *T. cameroni*, whereas in both *T. physellae* and *T. querquedullae* the males are almost double the size of the females. In *T. cameroni*, the caeca unite at the level of the prostate gland in the male and of the seminal receptacle in the female, whereas the caecal union in *T. physellae* is near the anterior end of the seminal vesicle and that in *T. querquedullae* is in the region of the gynecophoral fold just caudal of the genital pore. The caecal branches of *T. querquedullae* are described by McLeod, as uniting just posterior to the ovary, which is similar to the case of *T. cameroni*. However, its seminal receptacle is saclike, whereas the seminal receptacle in *T. cameroni* is a tubular structure. The adults described by McLeod are much shorter than those of *T. cameroni;* they have larger suckers in the male, a longer gynecophoral fold and a much greater number of testes.

Eggs

The eggs for the different species of schistosomes described so far are different. For this reason, McMullen and Beaver (8) identified their adult parasites from *Cercaria elvae* as *T. ocellata* described by Brumpt (1). The writer believes that eggs from different species may have a similarity in gross appearance but differ in some minor characters which are easily overlooked. The size and appearance of the egg of *T. cameroni* are apparently very similar to those of *T. physellae* and *T. querquedullae*. But a careful comparison of my material with the drawings of the eggs made by McMullen and Beaver and by McLeod reveals some characteristic features. The eggs of *T. cameroni* before they absorb water have gradually tapering poles (Figs. 10*a*, 11, and 12). The diagram of the egg of *T. physellae* apparently is also in this condition, since the miracidium is quite in contact with the egg shell. However, it has a very long blunt pole with the edges almost parallel and a very pointed, conical opposite pole; both poles are proportionately much longer. The egg as described by McLeod is apparently similar to that of *T. cameroni* (Fig. 10*c*), after water has been absorbed to some extent; it possesses two rather long poles. On the other hand, the more pointed conical pole of the eggs of *T. cameroni* always shows a characteristic appearance before or after the absorption of water, and the few exceptions show other variations and are obviously abnormal.

Cercariae

The cercaria of *T. cameroni* has a much longer body than *Cercaria physellae*. Both species have the same length of tail stem, but *Cercaria cameroni* has longer tail furcae, about two-thirds the length of the tail stem, whereas in *C. physellae* they are about one-half of the length of the tail stem. The head organ of *C. cameroni* is more slender. The ventral sucker is oval instead of round. The excretory bladder is separated, by a partition, into two, which differs from that described by Talbot (15) for *C. physellae*. Above all, one of the most important characters between species which has been emphasized by many authors is the difference in activity. According to Cort and Talbot (2) these cercariae *physellae*

> ". . . have a tendency to sink to the bottom of the container where they become attached. Occasionally they attach themselves to the side of the bottle but always near the bottom, and in relation to the source of light. In fact there is nothing in this activity that suggests any reaction to light either positive or negative. When at rest they assume a characteristic position with the ventral sucker attached and anterior part of the body in contact with the surface".

C. cameroni, like some of the species of the *Ocellata* group, attach to the surface with the ventral sucker and the pre- and post-acetabular regions bent away. They also differ from *C. physellae* in being positively phototactic, attaching themselves to the lighted side near the surface of the container.

The lengths of the body, tail stem, and the tail furcae, are closer to those of *C. elvae* Miller, 1923 (9) (redescribed by Talbot, 1936 (15)). But the attachment of these two species is different; according to Cort and Talbot (2),

> ". . . *C. elvae* attach themselves to the surface of the glass by the anterior tip and the protruded ventral sucker with the body between arched away from the furcae. The tail stem is turned back over the body and forms an acute angle . . .".

The characteristic attachment of the cercariae to the container resembles three species reported by Szidat: *C. neocellata* and *C. parocellata* (1942) and *C. pseudocellata* (1934). *C. neocellata* appears to be a smaller cercaria. Its snail host is *Limnaea palustris*. Moreover, Szidat infected ducks with *C. neocellata* and recovered eggs from one of the duck's faeces like those described by Brumpt (1) for his parasite, *T. ocellata*.

Cercaria pseudocellata is a slightly larger species and with a much longer tail stem and furcae. Its snail host is *Limnaea palustris*.

C. cameroni resembles *C. parocellata* in body length, but is more slender, its head organ is longer and more slender, the distance from the ventral sucker to the posterior end is much shorter, and the tail stem is also slightly shorter.

For these reasons, this species is regarded as new and the name *T. cameroni* is assigned to it.

For purpose of comparison, part of Szidat's Table I (13) for the measurement of *Cercaria neocellata*, *C. parocellata*, and *C. pseudocellata* and that for *Cercaria physellae* are reproduced in Table II. The measurements for the adults of *Trichobilharzia cameroni*, *T. physellae*, and *T. querquedullae* are shown in Table III.

TABLE II

MEASUREMENTS OF CERCARIAE, MM.

	Cercaria neocellata	*C. parocellata*	*C. pseudocellata*	*C. physellae*
Length of body	0.27	0.34	0.37	0.265 ± 0.0084
Width of body	0.055	0.07	0.08	0.060 ± 0.0046
Head organ length/width	0.085/0.04	0.09/0.05	0.11/0.06	0.095 ± 0.0050 by 0.039 ± 0.0020
Ventral sucker length/width	0.026	0.035	0.045	0.029 ± 0.0024
Distance of ventral sucker to posterior end of body	0.1	0.125	0.17	0.080 ± 0.0052
Tail stem length/width	0.36-0.39/0.036	0.4-0.44/0.04	0.58/0.05	0.374 ± 0.0106 by 0.040 ± 0.0036
Tail furca length/width	0.23/0.02	0.24/0.025	0.31/0.028	0.196 ± 0.0078 by 0.032 ± 0.0009

TABLE III

MEASUREMENTS OF MALES AND FEMALES

	Trichobilharzia cameroni	*T. physellae* (McMullen and Beaver)	*Pseudobilharziella querquedulae* (McLeod)
Males			
Body length, mm.	3.18-5.71	Up to 7.5	3.7
Body width, mm.	0.063-0.097	—	0.150
Oral sucker	31-40 × 38-47μ	24-28 × 28-40μ	56 × 64μ
Ventral sucker	31-49 × 49-51μ	16-32μ diam.	73μ (immature)
Distance between oral and ventral suckers, mm.	0.226-0.370	0.16-0.34	0.274 from anterior end
Distance between ventral sucker and gynecophoral fold	0.230-0.536, mm.	0.14-0.34 mm.	678μ from anterior end
Testes, number	About 70-110	96-160	210-240
Length of gynecophoral fold, mm.	0.212-0.345	0.10-0.19 × 0.056-0.080	0.375 (immature)
Intestinal reunion	Level of prostate gland	Near anterior end of seminal vesicle (about equal distance as intestinal bifurcation from acetabulum)	In region of gynecophoral fold just caudad of genital pore
Females			
Body			
Length	3.82-4.94 mm.	Up to 4.4 mm.	1.86 mm.
Width	0.052-0.059 mm.	0.040-0.080 mm.	60μ
Oral sucker, mm.	0.025-0.034 × 0.032-0.040	0.024 × 0.024 to 0.044 × 0.028	0.034
Acetabulum, mm.	0.025-0.038 long × 0.030-0.047 wide	0.024-0.032 to 0.040 protruded	0.036
Distance between anterior end and acetabulum, mm.	0.213-0.342	0.2-0.3	0.250
Distance between acetabulum and ovary, mm.	0.179-0.250	0.1-0.3	0.170
Ovary			
Length, mm.	0.287-0.358	0.09-0.20	0.120 (over-all)
Width, mm.	0.016-0.033	0.020-0.072	0.056
Seminal receptacle			
Length, mm.	0.072-0.126		
Width, mm.	0.018-0.042		
Eggs			
Saline	57 × 147μ to 73 × 181μ 63 × 212μ	0.170 × 0.065 to 0.250 × 0.080	0.217 × 0.076
Water	66 × 198μ to 77 × 253μ 80 × 240μ		
Cecal reunion	At level of seminal receptacle	Posterior to seminal receptacle	
Papilla of Laurer canal	Just in front seminal receptacle	Just back of ovary	Just posterior to ovary

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STUDIES ON DIPTEROUS PARASITES OF THE SPRUCE BUDWORM, *CHORISTONEURA FUMIFERANA* (CLEM.) (LEPIDOPTERA: TORTRICIDAE)

I. *SARCOPHAGA ALDRICHI* PARK. (DIPTERA: SARCOPHAGIDAE)[1]

By A. P. Arthur[2] and H. C. Coppel[2]

Abstract

Sarcophaga aldrichi Park. is a native parasite which has been reared continually, though in small numbers, from *Choristoneura fumiferana* (Clem.) in British Columbia. It is also an important parasite of *Malacosoma disstria* Hbn. and is apparently widely distributed throughout North America. The parasite can be reared in the laboratory on pork liver, or on a mixture of liver and fish, from larvae deposited on the rearing medium by ovoviviparous females. The total time required for development from deposition of the larvae to the beginning of puparial formation was five to eight days. The puparia were formed 7 to 44 days later. No adults emerged without a period of diapause, which normally lasted throughout the winter months. The distinguishing characters of the immature stages are described. The methods used in rearing spruce budworm pupae for obtaining parasites are outlined.

Introduction

The natural control of the spruce budworm, *Choristoneura fumiferana* (Clem.), in British Columbia has been studied by officers of the Belleville laboratory since 1943. According to Wilkes, Coppel, and Mathers (28), some 45 species of insects have been recorded as parasites of the budworm in British Columbia. McGugan (20) listed some 70 parasitic species for North America. These data invariably indicate that although, of the primary parasites, the Hymenoptera outnumber the Diptera by two to one, the two orders are approximately equal in importance.

The sarcophagid parasite *Sarcophaga aldrichi* Park. has been recorded annually from budworm collections in British Columbia since 1948. Specimens recorded as *Sarcophaga* sp. in 1947 and probably those in 1943 to 1946 are of *S. aldrichi*.

The present paper is the first report on studies being conducted to provide biological and descriptive data for use in the identification of the dipterous parasites in all stages of their development. The techniques and equipment involved in handling the budworm host material collected in British Columbia and reared in Belleville are described. These methods make it possible to provide parent parasite stocks for laboratory studies on all dipterous parasites of the spruce budworm. Considerable field data have been accumulated on the life history and habits of *S. aldrichi* (e.g. Hodson (13, 14)). This paper concerns a study parallel to Hodson's under laboratory conditions, and any variations from previous investigations are indicated.

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Contribution No. 3080, Division of Entomology, Science Service, Department of Agriculture, Ottawa, Canada. The greater part of this paper was submitted by Arthur in partial fulfillment of the requirements for the degree of Master of Science in forestry and conservation at the University of Michigan, 1951.
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Hosts

Sarcophaga aldrichi appears to be limited to lepidopterous hosts. It has been obtained in the largest numbers from the forest tent caterpillar, *M. disstria*. Emergence from *M. disstria* was first recorded by Caesar (5). Further records from this host were reported by Brown (4), Felt and Bromley (7), and Hodson (13, 14). It has also been reared from the gypsy moth, *Porthetria dispar* (L.), by Parker (21); the satin moth, *Stilpnotia salicis* (L.), by Glendenning (8); and the spruce budworm, *C. fumiferana*, by Wilkes *et al.* (27).

Distribution

The literature indicates that *S. aldrichi* is Nearctic in distribution. A map showing its distribution is given as Fig. 1, the records having been taken from Caesar (5), Parker (21), Aldrich (1), Johnson (16), Leonard (19), Glendenning (8), Winn and Beaulieu (29), Hallock (11), Strickland (25), Hodson (13, 14), and Felt and Bromley (7). Further data were obtained through personal correspondence with G. Styskal, Grosse Isle, Michigan; R. Dodge, Atlanta, Georgia; and G. E. Shewell, Division of Entomology, Ottawa, Ontario. In all probability, the distribution of *S. aldrichi* is wider than is shown in the figure, since *M. disstria*, according to Craighead (6), is distributed throughout the greater part of Canada and the United States.

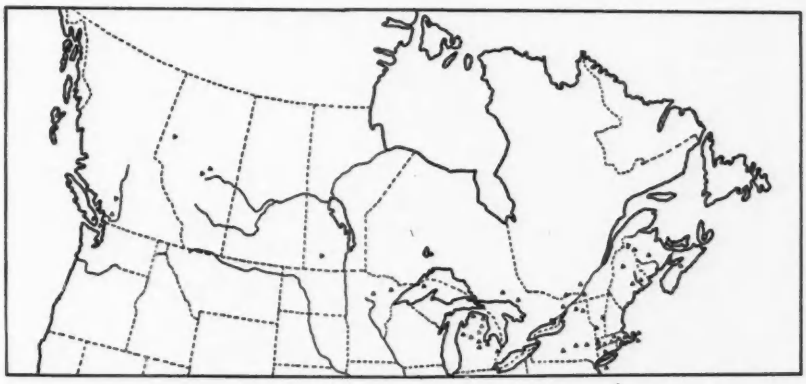


FIG. 1. The recorded distribution of *S. aldrichi* in North America.

Materials and Methods

Choristoneura fumiferana

The spruce budworm in the larval and pupal stages were collected near Lillooet, B.C. As pupae formed they were mixed with fine, dry sawdust and sent by air express to the Belleville laboratory. On arrival at Belleville the pupae were sifted from the sawdust, counted, and incubated for parasite emergence. A small amount of excelsior was placed in a shallow, galvanized

metal tray (Fig. 2, *b*), and covered with a sheet of white blotting paper. The pupae were then placed on the blotting paper to a depth of approximately one inch. The blotting paper absorbed excess moisture accumulated in transit. The trays of pupae were stacked inside cheesecloth-covered cages fitted with removable transparent plastic fronts (Fig. 3). Corks with right-angled grooves cut into the tops were set on the corners of the trays. The corks held the trays apart, allowed adults to escape, and provided aeration for the cage unit. The incubation room was maintained at a constant temperature of 22 ± 1° C. and 80% relative humidity. The adult parasites were removed daily, separated according to sex, and determined to species. After the adults were removed the cages were opened and the pupae closely examined. The mature dipterous larvae found among the budworm pupae were placed in metal cans containing damp sawdust. Any puparia which had formed were placed in the grooves of the corrugated cardboard. These sheets of cardboard were then placed on wooden trays in cheesecloth-covered cages (Fig. 3). The ends of the wooden trays (Fig. 2, *a*) were made higher than the sides, thus providing a space through which the adult flies could escape.

Sarcophaga aldrichi

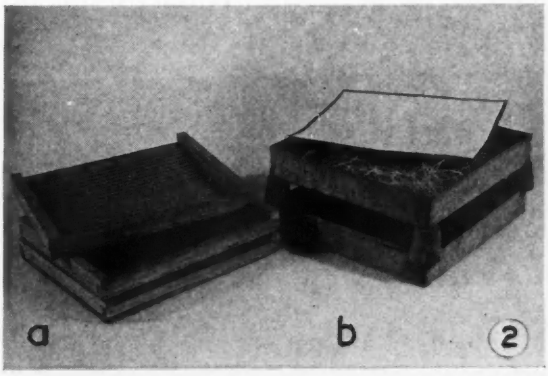
The puparia of *S. aldrichi* were mixed with sawdust and placed in glass vials (4 × ¾ in.) tightly plugged with cotton. They were then incubated at 7 ± 1° C. and 80 to 90% R.H. for two weeks. The material was stored for the winter at 1° C. and 80 to 90% R.H.

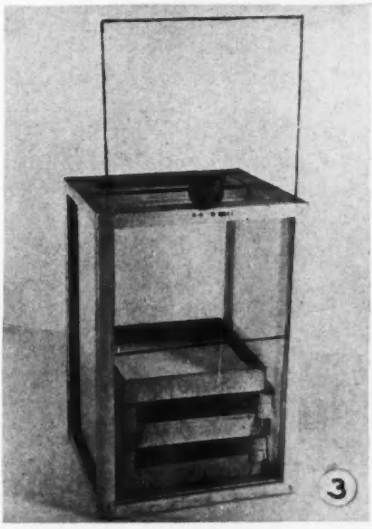
In 1950 the puparia were removed from cold storage on June 1 and incubated at 21 ± 1° C. and 60% R.H. They were sprayed twice daily with a fine mist of water to prevent excessive drying. Upon emergence, the adults were placed in cages (Plate I, Fig. 4). The cages were sprayed with water and the flies fed daily on a 10% aqueous solution of honey, made available to the flies by soaking a small pad of dental cotton in the solution and placing it in the cage on a square of waxed paper. Raisins were pinned to the backs of the cages to provide the parasites with proteins. At the end of each day the cages were taken from the rearing room at 21° C. and stored at 15° C. during the night.

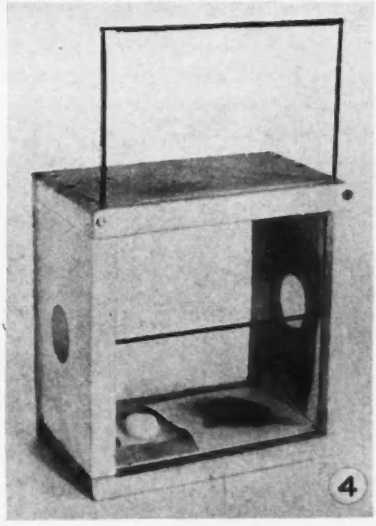
The flies mated when a newly emerged female was placed in a cage containing one or more males, one day old or older. The mating pairs were isolated and the females, after separation, were placed in special cages for oviposition.

A nutrient medium, consisting of one part salmon and three parts pork liver, developed by House and Traer (15) to rear *Pseudosarcophaga affinis* (Fall.), was suitable for rearing *S. aldrichi*. Later experiments showed that fresh pork liver alone was equally satisfactory. To promote larviposition a small portion of this medium was placed on a square of waxed paper and placed in the cage. The cages were examined hourly and the larvae removed and placed on the nutrient medium in the rearing dishes. Since records on larval development were desired, the larvae were reared individually in Stender dishes (37 × 20 mm. to 62 × 35 mm., o.d.). As the larvae developed, the

PLATE I







FIGS. 2-4. 2. Trays used for incubating (*a*) dipterous puparia, (*b*) budworm pupae. 3. A cage containing trays used for rearing budworm pupae. 4. A cage in which mated females are kept. It contains both food for the adults and liver on which eggs and larvae are deposited.

food was replenished from time to time. Measurements of larvae in early and late stages of each instar were made in the following manner. The larvae were anesthetized and placed in position on a microscope slide. By means of a compound microscope fitted with a prism, the magnified image of the larva was projected on a vertical surface and its outline traced on paper. The slide holding the larva was then removed and a slide micrometer was substituted. The developing larvae were kept in a room with temperature and humidity conditions similar to those used for incubating the puparia. As the larvae matured they were removed from the rearing dishes and placed in paper cups containing moist sawdust to form their puparia. After the puparia were formed they were placed in vials and stored for overwintering. The lengths and widths of the laboratory-reared puparia were measured with micrometer-calipers.

Life History

Adult Development

Adults of *S. aldrichi* began to emerge 11 days after the puparia had been placed in incubation, and continued to emerge for six days. Emergence of males began and came to a peak approximately two days before that of females (Fig. 5).

The literature indicates that the adult flies live for long periods in the field. Hallock (12) states that *S. aldrichi* is on the wing in New York State from late May until the middle of July. Styskal (in litt.) states that adults have been captured in central Michigan from May 18 to July 5, and at Isle Royale, north of the Michigan mainland, as late as Aug. 7. Similarly, in the laboratory the adult parasites lived for long periods. The length of life of *S. aldrichi*

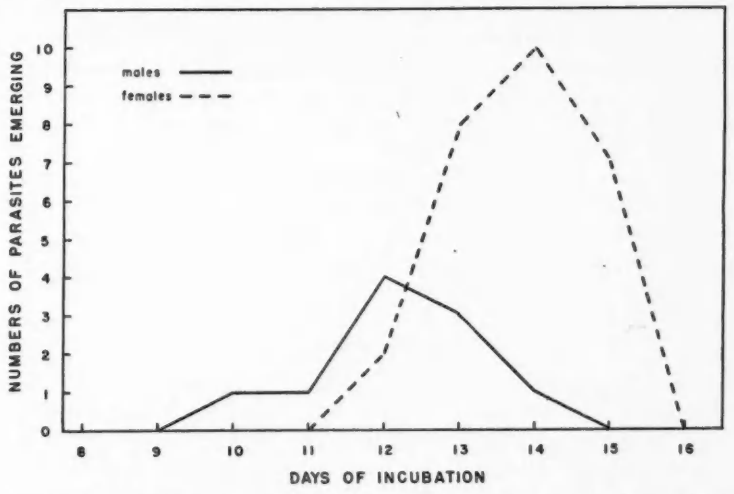


FIG. 5. Rates of emergence of males and females of *S. aldrichi* obtained in the laboratory during 1950.

reared under laboratory conditions was 46 days for males and 67 days for unmated females. Fifteen mated females had a life span from 23 to 119 days.

Under field conditions mating takes place as soon as the first females of *S. aldrichi* appear (Hodson (13)). Similarly, in the laboratory, mating took place readily if newly emerged females were placed with males one day or more old. The flies remain in coitus for varying lengths of time. Some pairs separated after 12 to 16 min., whereas others remained together for at least 2.5 hr.

The prelarviposition period of *S. aldrichi* is longer than that of many insect parasites. Hodson (13) found that in the field this period lasted from 20 to 26 days. In the laboratory this period was between 21 and 30 days. After once beginning larviposition, the females deposited intermittently until death, a period of 83 days in the case of one female.

Larval Development

First instar larvae molted at 32 to 51 hr. after deposition. The second molt took place at 60 to 85 hr. The larvae became fully fed in five to eight days. One third-instar larva removed from the medium when only three days old formed its puparium 18 days later; it was, however, much smaller than average. In all probability this larva had not finished feeding, since larvae removed from the medium on or after the fifth day formed puparia of normal size, usually in 7 to 44 days.

Puparial Development

During the summers of 1948, 1949, and 1950, 895 puparia of *S. aldrichi* were obtained from spruce budworm collections (Wilkes *et al.* (27)). From these only one adult emerged the same year. This indicates that the puparia enter a dormant stage which is broken by exposure to a period of low temperature. Hodson (13), however, cited an unpublished paper by R. F. Anderson which does not agree with the above. Anderson indicated that when *S. aldrichi* "develops parasitically" there is but one generation per year, whereas there are two when it feeds on carrion. However, Dr. Anderson (in litt.), Forestry Department, Duke University, Durham, N.C., indicated that he has never since been able to obtain two generations in one year. In experiments at the Belleville laboratory, both Ontario and British Columbia stocks were reared on liver for one or more generations. As only one generation was obtained each year, it is assumed that a second generation does not normally occur even when the parasite feeds on carrion.

Habits

The literature indicates that the adult is a strong and active flier. Felt and Bromley (7) made the following comment on this parasite during the outbreak of the forest tent caterpillar in the Livingstone Manor region of New York State: "They swarmed over livestock, organic material, exposed dishes, drinking cups, and other utensils, as well as human beings, thus constituting a general nuisance".

The mating habits of *S. aldrichi* were carefully observed in the laboratory, and are somewhat similar to those described by Baldwin and Coppel (2) for *Phorocera hamata* A. & W. The act of mating is not necessarily preceded by a period of courtship. The prothoracic legs of the male are used usually to grasp the anterior margins of the female's wings near their bases. The mesothoracic and metathoracic legs usually rest on the surface on which the female is standing. The dorsum of the male's thorax is at approximately a 60° angle with this surface, whereas the abdomen is nearly vertical. There seems to be very little movement once the pair are in position, although at the beginning the female often attempts to dislodge the male.

Hodson (13) showed that *S. aldrichi* is a prepupal and pupal parasite of *M. disstria* and that in the field the end of the prelarviposition period coincides with the host's cocoon-spinning period. The small numbers of *S. aldrichi* obtained from collections of larvae of the spruce budworm from British Columbia (Table I) when compared with the larger numbers obtained from pupal collections confirm Hodson's findings.

The females of *S. aldrichi* normally deposit living larvae, although some females may deposit eggs only. The eggs laid by the oviparous females failed to develop in the laboratory unless the mouth hooks were visible. Knipling (18) and Smith (23) found that some sarcophagid eggs are prematurely deposited and produce larvae only if kept warm and moist. Females of *S. aldrichi* deposited eggs and/or larvae intermittently. There were periods during which a female would deposit from 10 to 25 eggs or larvae in two to five days, alternating with periods of intrauterine incubation during which no

TABLE I

NUMBERS OF *S. aldrichi* OBTAINED FROM THE SPRUCE BUDWORM COLLECTED IN BRITISH COLUMBIA

Year	No. of larvae collected	No. of *S. aldrichi* reared	% of larvae parasitized	No. of pupae collected	No. of *S. aldrichi* reared	% of pupae parastized
Mount McLean						
1948	25,761	3	0.010	7996	1	0.010
1949	22,844	1	0.004	13,225	4	0.030
1950	18,259	3	0.016	39,135	82	0.210
Total	66,864	7		60,356	87	
Fountain Valley						
1948	24,879	3	0.010	342,915	225	0.065
1949	10,163	2	0.020	532,006	103	0.019
1950	35,963	11	0.031	207,479	452	0.218
Total	71,005	16		1,082,400	780	

deposition occurred (Fig. 6). The females on which individual records were maintained deposited an average of 13.7 larvae and 16.6 eggs per female. Those eggs and larvae were consistently deposited in the bottom area of the cage, near the nutrient medium, to which the larvae usually made their way. When fully developed eggs are deposited, the larvae escape by catching their mouth hooks into the anterior part of the chorion and tearing their way out. This can be readily observed through the semitransparent chorion.

When two newly hatched larvae were deposited near one another and accidentally met, often one would kill and partially devour the other. This cannibalistic habit was not observed after the larvae had once eaten other food. During feeding, the larvae were usually buried in the medium with only the posterior portion of the body visible. Even if the medium became moist and pools of liquid collected on its surface, the larvae were still able to feed. The shape of the larva and general covering of the body appear to inhibit wetting and thus permit the larva to draw its entire body beneath the

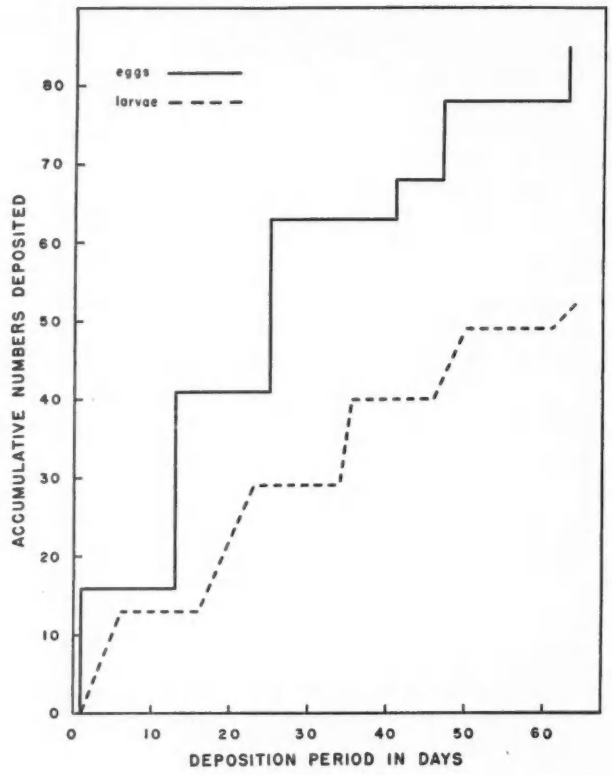


FIG. 6. Deposition of eggs and larvae by females of *S. aldrichi* under laboratory conditions, showing intermittent reproduction.

surface of the fluid. A dimple-like depression forms in the medium at the posterior end of the larva, allowing the spiracles to remain in contact with the air. If, however, the larva penetrates too far into the food medium, the surface tension is broken. The larva then closes the posterior cavity, preventing the entrance of fluid into the tracheal system. As air is required the larva rears its posterior end out of the liquid and simultaneously opens the posterior cavity.

It is possible to predict the onset of molting approximately 6.5 hr. before it takes place. At this time the posterior spiracles of the succeeding instar become faintly visible through the cuticula, anterior to the functional spiracles. They become plainly visible before the molt. At the same time, the cephaloskeleton of the succeeding instar appears directly dorsad to that of the present instar. The two cephaloskeletons are in approximately the same relative positions as the two sets shown by Smith (23) in *Sarcophaga securifera* Vill. Molting occurred usually on the surface of the medium. The larva alternately expanded and contracted the anterior part of the body by forcing the body fluids in and out of this region. The presence of a milky-white molting fluid was noted just under the cuticula in the middorsal area, where the split later occurred. After the contractions had continued for a few seconds there was a final, sudden flow of fluids into the anterior part of the body. As a result a split occurred in the outer cuticula along the middorsal line from a point immediately above the oral region to the second or third thoracic segment. The larva then raised its head out of the exuviae, twisted slightly to one side, and thus lengthened the slit. By burrowing into the medium, the larva left the exuviae behind.

The feeding habits of the second and third instar larvae were similar to those of the first. Fully fed larvae feeding in nutrient medium which remained moist would leave the food and cling to the rearing dish. If the medium became dry the larva would crawl between it and the bottom of the dish and lie quiescent. Similar responses to moisture were observed in the field by Hodson (13), where under moist, or normal, conditions the fully fed larvae left their host and entered the ground to form their puparia. He noted, however, that during the dry summer of 1936 most of the larvae remained in the host cocoons for more than a month, and left the cocoons to enter the ground only after it had rained for several days.

Descriptions of Stages

Egg

The egg is macrotype, pearly white, and elongate–oval. It is of uniform width in the posterior half and narrows slightly toward the anterior end. In lateral view, the dorsal surface is slightly convex and the ventral surface slightly concave, giving the egg a curved appearance (Fig. 7). It is subcircular in cross section. It has a membranous, semitransparent chorion. Reticulate markings (Fig. 8) are present, but were observed only when a single thickness of the chorion was studied under the microscope.

Larva

In the study of parasitic species it is important to be able to identify the immature stages as well as the adults. Although dipterists in general have given little attention to the characters by which the species can be distinguished in the larval stage, careful study by a few of the more recent authors such as Root (22), Greene (9), Knipling (18), and Smith and Finlayson (24) indicates that a large majority can be specifically determined.

The nomenclature used for the various sclerites of the cephaloskeleton is that of Hall (10). Measurements for all the immature stages of *S. aldrichi* reared on an artificial medium are shown in Table II.

TABLE II

MEASUREMENTS OF THE IMMATURE STAGES OF *S. aldrichi* REARED ON AN ARTIFICIAL MEDIUM

Stage	No. examined	Length, mm.		Distance from anterior end	Width, mm.	
		Average	Range		Average	Range
Egg	15	2.71	2.45– 2.85	—	0.72	0.65–0.80
1st instar larva						
Newly hatched	10	4.20	3.80– 4.48	2.00	0.82	0.75–0.87
Before first molt	6	6.45	5.30– 7.25	3.50	1.43	1.22–1.43
2nd instar larva						
Early	6	7.83	7.17– 9.20	4.00	1.91	1.65–2.30
Before second molt	8	11.16	10.15–11.80	5.00	2.74	2.45–3.00
3rd instar larva						
Early	6	10.23	9.00–11.20	5.00	3.31	3.10–3.50
Fully fed	10	13.08	12.10–14.30	6.00	3.93	3.35–4.40
Puparium	10	11.19	10.82–12.70	Widest point	5.00	4.45–5.55

First Instar Larva

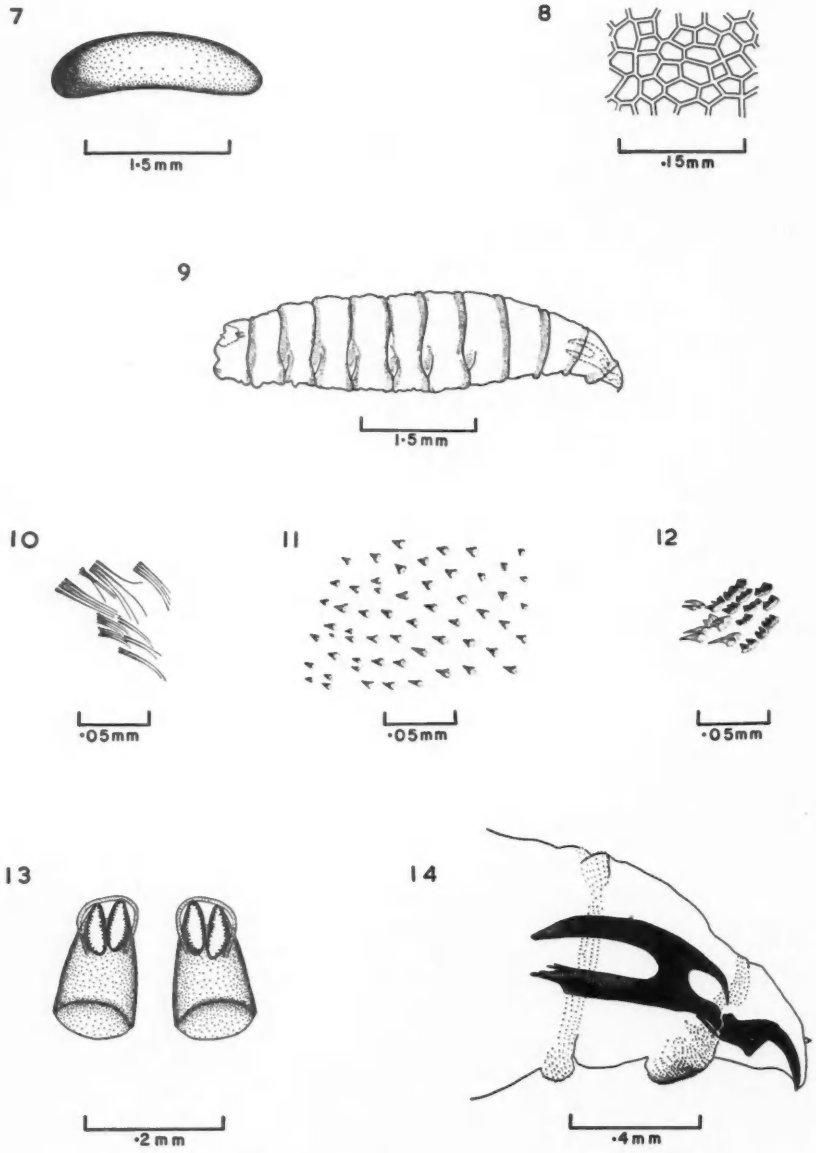
The first instar larva is slender and tapers both anteriorly and posteriorly (Fig. 9). The body consists of 12 segments: three thoracic, eight abdominal, and one small, conical pseudocephalon. The cuticle is semitransparent, so that the whitish internal organs and the heavily sclerotized cephaloskeleton are clearly visible. The integument is armed with spines in bands of closely approximated rows. These bands are near either the anterior or posterior borders of the segments (Fig. 3 and Table III). The spines on the anterior border are recurved posteriorly, whereas those on the posterior border are recurved anteriorly. The band of spines on the anterior border of the prothoracic segment (second apparent segment) is widest ventrally. It is composed of a number of heavily sclerotized spines with two or three blackened tips projecting from a common base (Fig. 12). The spines on the other parts of the larva are simple, similar to those shown in Fig. 11. The bands of spines on the anterior borders of the two remaining thoracic segments and the

TABLE III

APPROXIMATE NUMBERS OF ROWS OF SPINES ON THE DORSAL AND VENTRAL REGIONS OF THE SEGMENTS OF *S. aldrichi* LARVAE

Stage	Band	Position	Segments											
			1	2	3	4	5	6	7	8	9	10	11	12
I	Anterior	Dorsal	0	8– 9	9–11	11–12	9–10	7– 9	7– 8	5– 7	4– 6	0– 6	0– 4	0
		Ventral	0	11–13	8–10	7– 9	8–10* and 4–6 + 2–5	13–15	11–13 and 7–9 + 3–5	13–15	11–12	10–12	8–10	0–8
	Posterior	Dorsal	0	0	0	0	0	0	2– 5	4– 7	6– 8	7 –9	8– 9	0
		Ventral	0	0	0	0	0	0– 5	0– 5	3– 8	6– 8	5– 7	5– 8	0
II	Anterior	Dorsal	0	6– 8	9–10	8–11	6– 8	4– 6	3– 6	1– 5	0– 5	0– 5	0– 5	
		Ventral	0	12–18	6– 8	6– 9	8–10 and 3–4 + 3–4	6 –9 and 6–8 + 2–4	8 and 6–8 + 2–3	5– 8 and 6–9 + 1–2	8– 9 and 7–8 + 1–3	7– 8 and 6–8 + 1–4	8–10 and 7–8 + 0–3	7 and 6–8 + 1–2
	Posterior	Dorsal	0	0	0	0	0	0	0– 4	1– 5	3– 5	2 –6	4– 7	3–5
		Ventral	0	0	0	0	0	2– 4	1– 5	2– 5	2– 5	3– 7	2– 4	0
III	Anterior	Dorsal	0	0– 6	9–12	8–11	8–11	4–7 + 0–3	3–6 + 0–2	3–5 + 0–3	2–5 + 0–2	0– 5	0– 5	0–4
		Ventral	0	14–16	5– 8	6– 8	8– 9 and 2–4 + 2–4	8– 9 and 3–4 + 1–3	7– 9 and 4–5 + 1–4	8–10 and 5–7 + 1–3	7– 9 and 4–7 + 1–3	6– 7 and 4–6 + 2–3	1– 8 and 3–5 + 1–4	5–7 + 1–2
	Posterior	Dorsal	0	0	0	0	0	0	0	0	0	0	0	0
		Ventral	0	0	0	0	0	0	0	0	0	0	0	0

** The band contains 8–10 rows of spines on the ventrolateral surface; but ventrally there are two bands, an anterior one containing 4–6 rows of spines and a posterior one containing 2–5 rows.*



FIGS. 7-14. 7. Egg, lateral view. 8. Egg pattern. 9. First instar larva, showing arrangement of spines. 10. Spines from the rim of the posterior cavity. 11. Section of the spinal band on the pleural area of the second thoracic segment. 12. Spines from the ventral surface of the spinal band on the prothoracic segment. 13. Posterior respiratory apparatus of first instar larva. 14. Cephaloskeleton of first instar larva.

first abdominal segment are of approximately uniform width throughout and composed of 7 to 11 rows. The width of the bands on the anterior borders of the remaining abdominal segments becomes narrower on each succeeding segment. These bands are broken opposite the lateral fusiform pads on abdominal segments two to seven. They are also interrupted by spineless areas on the dorsal surfaces of the fifth and sixth abdominal segments. Posterior to these segments, the bands on the anterior borders are represented only on the ventral and ventrolateral surfaces. The posterior border of the second abdominal segment has a small number of anteriorly directed spines on the dorsal and ventral surfaces. These increase in number posteriorly, forming discontinuous bands of increasing width on the posterior borders of abdominal segments three to seven. The last abdominal segment is encircled by scattered, comblike rows of long, slender spines (Fig. 11) near the rim of the posterior cavity. A few scattered spines are also present on the anal lobes.

The first instar larva is metapneustic. The posterior spiracles (Fig. 13) are in a deep oval cavity on the posterodorsal area of the last segment. Each spiracle contains two ovate slits, which are nearly united near their ventral margins. These slits are subparallel to each other and approximately parallel to the median sagittal plane of the larva. The distance separating the two spiracles is approximately equal to the length of the outer slit.

There are a number of white, fleshy processes on the border of the posterior cavity. Only three pairs appear to be present in this instar. These are an inner and an outer pair on the dorsal border, and an inner pair on the ventral border of the cavity. A pair of anal tubercles is situated ventral and slightly posterior to the latter pair.

No spines are visible on the pseudocephalon of the first instar larva, but it bears two groups of sensory organs at the anterior end. These groups of sense organs are referred to as the antennomaxillary complex by Keilen (17). The antennae are two-segmented and approximately 30μ long. The basal segment is about as long as broad; the second is nearly twice as long as broad and conical in shape. Ventrad of each antenna are the maxillary palpi, which appear as sclerotized circular, or oval, rings. The smaller pair is nearly circular in outline and slightly closer to the antennae than the other pair. Each is approximately 5μ in diameter. The larger pair is oval in outline and, on the specimens measured, the greatest diameter was approximately 20μ.

The mouth is ventral and slightly posterior to the antennomaxillary complex. The paired mouth hooks of the cephaloskeleton protrude from the oral cavity as shown in Fig. 14. They articulate basally with the pharyngeal sclerite. This sclerite is composed of two pairs of nearly parallel cornua, one pair dorsal and the other ventral. Both pairs are less heavily sclerotized toward their posterior extremities, although this was less marked in the ventral cornua of some specimens. The anterior ends of the dorsal cornua are connected to form a curved process, the dorsopharyngeal sclerite. The ventral cornua also extend anteriorly each articulating with the paired labial sclerites, or mouth

hooks. The labial sclerites are comparatively massive structures; their somewhat enlarged, subrectangular bases forming their articulating faces with the hypostomal sclerite. The decurved hooks extend from the anterodorsal part of each basal sclerite. Immediately below the hypostomal sclerite is a small infrahypostomal sclerite, H-shaped as seen in ventral view, with the longer arms extending anteriorly. No dental sclerite was seen in this instar.

Second Instar Larva

The body of the second instar larva is similar in shape to that of the first instar except that it is larger and slightly more robust.

In general the arrangement of the spines on the borders of the segments of the second instar larva is similar to that described for the first, and is shown in Table III. The following differences, however, may be noted. There are lateral breaks in the bands of spines on segments two and three. No spines are found on the dorsoposterior surface of segment six. The spines on the posterior bands of segments seven and eight are not continuous with those on the lateral fusiform pads. The anterior bands of spines on segments 9 and 10 are wider than those in the first instar. There are a few spines on the dorsolateral surface of the anterior part of segment 11; these are not present in the first instar larva.

The second instar has a pair of anterior spiracles, although they may not be functional. They are fan-shaped; each is on the pleural area near the posterior margin of the prothoracic segment. A maximum of 72 lobes was counted on each anterior spiracle of mounted second instar larvae.

The posterior spiracles (Fig. 15) are paired, each with two subparallel slits. The slits are partly surrounded by a ringlike peritreme, which is incomplete ventrad of the slitlike openings. The average distance separating the posterior spiracular plates is 11 mm.

There are both an inner and an outer pair of fleshy processes on the dorsal border of the posterior respiratory cavity. On the ventral border of the cavity there are both a well-defined inner pair of processes and a poorly defined outer pair.

The cephaloskeleton (Fig. 16) can be easily separated from that of the first instar because the caudal portion of each dorsal cornu is deeply divided by an anteriorly directed notch. A lightly sclerotized area is present on each dorsal cornu. This extends posteriorly along the dorsal margin in the form of a band extending from the anterior edge, just ventrad of the dorsopharyngeal sclerite. The articulation between the anterior surface of each ventral cornu and the hypostomal sclerite can be seen in lateral view. Each ventral cornu extends anteriorly, dorsal to the hypostomal sclerite, forming a process known as the parastomal sclerite. In lateral view the hypostomal sclerite is subtriangular, the ventral portion of each lateral area being connected by a narrow bar. The labial sclerites are similar in shape to those found in the first instar larva. However, the posterodorsal process is more prominent. Immediately below the hypostomal sclerite is a small, H-shaped infrahypostomal sclerite,

its longer arms extending anteriorly. A pair of comma-shaped dental sclerites lie ventral to the basal portion of each labial sclerite. Immediately posterior to the oral opening are a group of posteriorly recurved spines whose bases are continuous with sclerotized bars. The bars extend dorsally toward the cephaloskeleton. The position of the spines suggests that they are rasping organs supplementing the mouth hooks.

Third Instar Larva

The third instar larva (Fig. 17) is distinctly robust. It tapers toward both ends. The cuticle of a newly molted larva is milky-white in color and that of a fully fed larva slightly darker. The arrangement of the spines on the segmental borders is similar to that described for the first instar larva (Table III and Fig. 17). The following differences, however, may be noted. No spines are present on the posterior borders of the abdominal segments, except those of the lateral fusiform pads. There are a few spines on the dorsolateral surface of the anterior part of the eleventh segment. There are no spines on the lateral surface of the anterior border of the mesothoracic segment. The fine, hairlike spines (Fig. 18, *a*) that occur in comblike rows near the rim of the posterior cavity are not as long as those on the first instar larva. The bands on the segmental borders of the third instar contain compound spines, especially in the most posterior rows (Fig. 18, *e*), whereas those in the first instar are all simple. The compound spines on the ventral surface of the anterior border of the prothoracic segment are similar to those in the first instar (Fig. 18, *b* and *c*).

The third instar larva is amphipneustic. The anterior spiracles are similar to those in the second instar. Greene (9) esimates that each spiracle has about 80 small lobes, each having a single opening. The posterior spiracular plates each contain three subparallel slits (Fig. 19). Each plate is bordered by a peritreme, which is incomplete ventrad of the slits. The inner portion of the peritreme is nearly vertical and ends freely in a prominent knob. The button is lightly pigmented and clearly visible ventrad of the inner and middle slits.

There are three pairs of fleshy processes on the dorsal border of the posterior cavity. The middle and least prominent pair lies slightly anterior to the other two. On the lower border of the cavity there are also three paired processes. The middle pair extends in a vertical direction and is the most prominent. The other two pairs are poorly defined.

The cephaloskeleton (Fig. 20) is similar to that in the second instar larva; however, the dorsopharyngeal sclerite is much less prominent, and the dorsal portion of each dorsal cornu is less heavily sclerotized. The rasping organs (Fig. 18, *d*) are similar to those of the second instar.

Puparium

The puparium of *S. aldrichi* has been described by Greene (9). In his description of the posterior spiracles he stated that "between the first and second slit is a short yellow line which resembles a short slit". Study of

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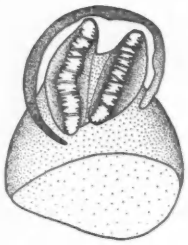

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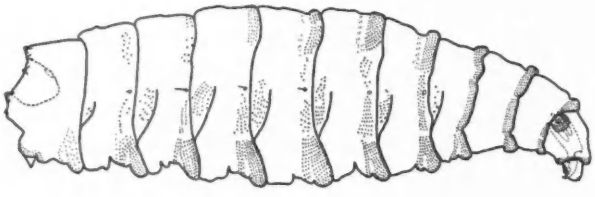

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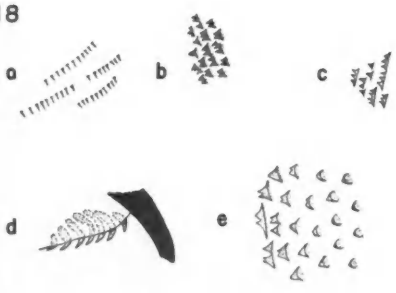

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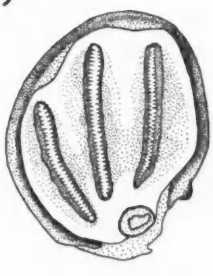

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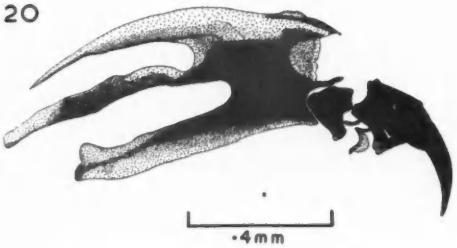

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48 puparia of *S. aldrichi* reared from the spruce budworm indicated that this "short yellow line" does not occur in all puparia. The line was absent in 23 puparia, on one plate only in 5 puparia, indistinct on 8 puparia, and as described by Greene in 12 puparia. Therefore, it is not as important as a distinguishing character as Greene's description would indicate.

Microscopic examination failed to locate either of the prothoracic cornicles in the puparium or any direct connection between the internal spiracle on the prothorax of the developing pupae and the puparial wall. Each internal spiracle contains large numbers of respiratory papillae arranged in branchlike fashion radiating from a central area (Fig. 21).

Adult

The adult male was named and fully described by Parker (21). The systematic position of this parasite has remained the same to the present time. Aldrich (1) includes Parker's description in his work, together with a figure illustrating the lateral view of the external male genitalia. He also gives a brief description of the female. Hallock (12) includes figures of the male genitalia, showing the basal apodeme, the ninth sternum, the pump sclerite, and a lateral view of the external genitalia. The chromosomes of *S. aldrichi* are described and illustrated by Boyes and Wilkes (3).

Male Reproductive System

The male reproductive system resembles that found in tachinids and is similar to that of *P. hamata* as described by Baldwin and Coppel (2). The paired testes are elongate, tapering, and heavily pigmented. The vasa differentia are of uniform diameter, the distal one-third being pigmented like the testes. Each vas deferens is slightly longer than the testes. The accessory glands are long and slender, of fairly uniform diameter, and approximately twice as long as those of *P. hamata*. These glands join the ejaculatory duct just posterior to the vasa deferentia. The ejaculatory duct enters a well-developed ejaculatory sac anterior to the penis.

Female Reproductive System

The reproductive system of the female is similar to that of *Chrysostomomyia crysostoma* Wied. as described by Townsend (26). Each of the two ovaries is made up of a relatively small number of ovarioles; the exact number is unknown as the ovarioles were not well differentiated in the mounted specimens examined. Each ovary is united to a common oviduct by a short lateral oviduct. The common oviduct is a long, looping tube much enlarged posteriorly to form the uterus. The three ovoid spermathecae are dark brown

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FIGS. 15-21. 15. Posterior respiratory spiracles and felt chamber of second instar larva. 16. Cephaloskeleton of second instar larva. 17. Third instar larva, showing the spinal pattern. 18. Spines of third instar larva: (*a*) near rim of posterior cavity, (*b*) on ventral surface of prothoracic segment, (*c*) on lateral surface of prothoracic segment, (*d*) posterior to oral opening, and (*e*) section of band on pleural area of second thoracic segment. 19. Posterior spiracular plate of third instar larva. 20. Cephaloskeleton of third instar larva. 21. Internal spiracle of puparium.

in color and are joined to the ventral surface of the uterus by long spermathecal ducts. Each duct enters the uterus separately, the median duct being slightly anterior to the two lateral ones. The distal ends of the ducts are surrounded by brown sclerotized rings for a distance equal to approximately one-half the length of each spermatheca. The middle three-fifths of each duct is about twice as large in diameter as the ends. There are two spherical, unsclerotized accessory glands joined to the uterus just posterior to the spermathecal ducts. The ducts of the accessory glands are shorter than those of the spermathecae with pronounced swellings near their proximal ends. The uterus has a highly extensile membranous wall perforated by numerous tracheae. The tracheae are so numerous that dissection and separation of the various organs is particularly difficult. The uterus of a gravid female may fill most of the abdominal cavity, and contain a maximum of 16 active first instar larvae.

Acknowledgments

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STUDIES ON DIPTEROUS PARASITES OF THE SPRUCE BUDWORM, *CHORISTONEURA FUMIFERANA* (CLEM.) (LEPIDOPTERA: TORTRICIDAE)

II. *PHRYXE PECOSENSIS* (TNS.) (DIPTERA: TACHINIDAE)[1]

By M. G. Maw[2] and H. C. Coppel[2]

Abstract

Phryxe pecosensis (Tns.) is a native parasite attacking several species of lepidopterous larvae. It has been reared consistently from collections of the spruce budworm made in British Columbia. The time required from egg deposition to the beginning of the formation of the puparium was 7 to 11 days, the female taking the longer period. The puparium was usually completed within a 24-hr. period and the adult emerged 9 to 11 days later. The life history and habits of the insect are described and the salient characters of the immature stages and the internal reproductive systems of the adults are illustrated and described.

Introduction

The tachinid parasite *Phryxe pecosensis* (Tns.) has been continuously obtained from collections of the spruce budworm made in British Columbia as part of the biological control program of the Belleville laboratory (Wilkes, Coppel, and Mathers (8)). McGugan (4), Raizenne (5), and Sellers (6) record the existence of the parasite in the eastern and western American states, and in the eastern and western provinces of Canada. Collections from Quebec and Newfoundland indicate that it is well represented in the parasite complex of those areas.

Sellers (6) brought together the data on the systematic position and synonymy of *P. pecosensis*. The recognition of *pecosensis* reduced the confusion that had, before 1927, existed in North America concerning the identity of *Phryxe vulgaris* (Fall.). Until 1927, these two tachinid species had been considered as one. The locality of the type species is the Pecos National Forest, New Mexico (Townsend (7)). It appears that *P. pecosensis* favors areas with a temperate summer rather than the more arid and central parts of North America.

From the hosts listed by Sellers (6), McGugan (4), and Raizenne (5), the lepidopterous families Geometridae and Tortricidae seem to be preferred by the parasite. Phalaenidae and Saturniidae are next in importance, followed by Pyralidae and Nymphalidae in the order given.

It was possible by using *Pieris rapae* (L.) as a host to rear several generations of this parasite in the laboratory and obtain the biological data for this paper.

[1] *Manuscript received April 2, 1953.*

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Descriptions of Stages

Adult

According to Sellers (6), Townsend's description of *Plagiophryxe pecosensis* stands as an adequate description of the species.

Egg

The egg (Fig. 1) is approximately 0.5 mm. in length and 0.2 mm. in width. It is macrotype and grayish white in color. In dorsal view the general form is oblong with the posterior end broader than the anterior, giving a bluntly tapering appearance. Dorsally, the chorion is moderately thick, with a distinct pattern of hexagonal markings on its surface; ventrally, it is thin, translucent, and flexible. The egg is flattened or concave on the ventral surface, depending upon the surface upon which it is deposited. No micropylar openings were observed with the magnification used. The egg is attached to the host with a mucilaginous substance which sometimes appears as a distinct pad.

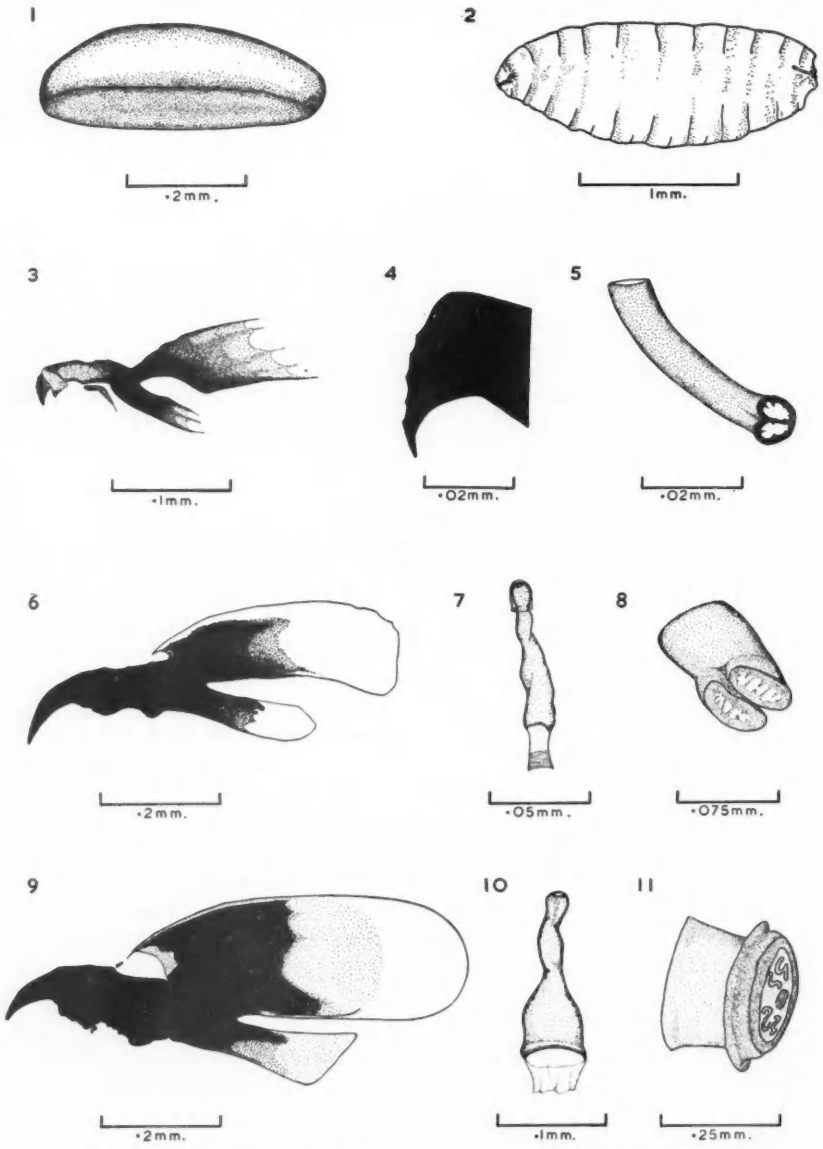
First Stage Larva

The first stage larva is typically tachiniform. The body tapers toward the anterior end and is somewhat truncate posteriorly (Fig. 2). It ranges in length from 0.7 mm. to 2.0 mm.; before escaping from the egg, it has an average length of 0.36 mm. The cuticula is colorless and semitransparent, and each segment bears an armature of spines arranged in discontinuous rows. The arrangement of the spines is shown in Table I. There is a slight variation in the spinal pattern between individuals. In general, however, spines on the anterior margins of the segments are directed posteriorly, and those on the posterior margins, anteriorly. Two conspicuous patches of strong, dark spines are found on the 11th segment, one dorsad and the other ventrad of the stigmatal openings.

The first stage larva is metapneustic. The two stigmata, situated on the last abdominal segment slightly above the longitudinal axis, are separated by a space equal to the width of one stigma. The stigmata are darkly pigmented on the lateral margins with the coloring graded to light brown on the median margins. Each stigma (Fig. 5) bears two openings with irregular inner margins. The openings are separated by a lightly pigmented septum. The felt chambers are approximately five times as long as wide. They are lightly pigmented and are directed dorsad from the stigmata at about a 30° angle.

The pseudocephalon bears the buccopharyngeal armature, a pair of sensory papillae, and a pair of preantennae on its anterolateral surfaces.

The buccopharyngeal apparatus (Fig. 3) is well developed and darkly pigmented. It is unjointed and the two wings are fused anteriorly to form a narrow, elongated V. The median hook turns abruptly downward. Its anterior margin is equipped with five coarse teeth (Fig. 4). On each side of the anterior hook is a lightly pigmented lateral plate. Its longitudinal axis is in an oblique plane, with the anterior parts projecting dorsoanteriorly.



FIGS. 1–11. *Phryxe pecosensis* (Tns.). 1. Egg, ventrolateral view. 2. First stage larva, showing arrangement of spines, positions of the posterior spiracles, and the buccopharyngeal armature. 3. Buccopharyngeal armature of first stage larva. 4. Tip of first stage mouth hook. 5. Posterior spiracle and felt chamber of first stage larva. 6. Buccopharyngeal armature of second stage larva. 7. Anterior spiracle and felt chamber of second stage larva. 8. Posterior spiracle and felt chamber of second stage larva. 9. Buccopharyngeal armature of third stage larva. 10. Anterior spiracle and felt chamber of third stage larva. 11. Posterior spiracle and felt chamber of third stage larva.

The intermediate portion of the apparatus is relatively long and moderately pigmented. There is a slight notch in the posterior portion and a small protuberance in the center of the ventral margin. In the dorsal margin, there is a depression opposite the ventral protuberance. The salivary plate is comparatively large and flat; the posterior half bends abruptly downward. The ventral wing is darkly pigmented for most of its length, with the posterior portions lightly pigmented. The dorsal and ventral margins are more or less parallel. The dorsal wing is narrow at its base and is heavily pigmented but widens abruptly, the posterior portions being less darkly pigmented except for the ventral margin. In dorsal view, both the dorsal and ventral wings converge slightly.

Second Stage Larva

The second stage larva averages 3.5 mm. in length, ranging from 2.8 mm. (newly molted larva) to 4.5 mm. (late second stage). In shape and color, the second stage larva resembles the first. The cuticular armature consists of bands of spines arranged in rows which are usually interrupted and confused. The spinal arrangement is shown in Table I. The spines are heavier and are more darkly pigmented than those of the first stage larva. Toward the posterior of the larva, the number of rows of spines on the anterior edge of each segment decreases progressively and the number of rows in the posterior bands increases. The posterior bands commence dorsally at segment VII, laterally and ventrally at segment VIII, and laterally at segment V except for conspicuous fusiform patches of three to five rows on segments VI, VII, and X. Dorsal spines on segments III to VII, when viewed from above, appear as fusiform patches with the greatest width at the middorsal line. A patch of very heavy spines is situated both dorsad and ventrad of the posterior stigmatal plate.

The posterior spiracles (Fig. 8) are lightly pigmented. Each spiracle has two irregular openings which appear to be bridged at irregular intervals by thin, lightly pigmented bars. The width of each spiracle is equal to twice the distance between the two spiracles. The felt chamber is lightly pigmented and is scarcely twice as long as broad.

The anterior spiracles (Fig. 7) are subcutaneous and are situated on the lateral aspect of the posterior margin of the first segment. Each appears as a grayish spot when the first segment is extended. The anterior end, bearing one orifice, is covered by a thin, semitransparent hood. The felt chamber is relatively long and lightly pigmented.

The pseudocephalon bears a pair of sensory organs and a pair of preantennae similar to, but more distinct than, those found in the first stage larva.

The buccopharyngeal armature (Fig. 6) is larger, darker, and thicker than that of the first stage larva. Instead of the single median hook there is a pair of heavy, curved mandibular hooks. The entire structure is apparently fused into a single unit, but in some specimens a thinning of the hook was detected, suggesting a point of fracture which would separate the anterior

TABLE I

APPROXIMATE NUMBERS OF ROWS OF SPINES ON THE DORSAL, LATERAL, AND VENTRAL REGIONS OF *P. pecosensis* LARVAE

Stage	Band	Position	Segments										
			I	II	III	IV	V	VI	VII	VIII	IX	X	XI
I	Anterior	Dorsal	4	4	3–4	3–4	3–4	3–4	3–4	2–3	3–4	2–4	2–3
		Lateral	4–5 +2*	5 +3**	4 +4	4	2–3	0	0	0–1	1–3	2–4	3
		Ventral	5–6	5–6	5	4	2	2–3	3	3	2	2	3
	Posterior	Dorsal	0	0	0	0	0	0	0	0	0	0	0
		Lateral	0	0	0	0	0	0	0	0	0	0	0
		Ventral	0	0	0	0	4	3–4	4	4	3	3	2–4
II	Anterior	Dorsal	6–7	6–7	4–5	4	2–4	1–4	1–4	1	0	0	0
		Lateral	5–6	5–7	4–5	3–4	0–1	0	3–5	0	0	1	0
		Ventral	6	10	6–8	5–8	2–3 +2	4 +3	2–3	2–3	3–4 +	3–4	4
	Posterior	Dorsal	0	0	0	0	0	0	0–1	2–3	4–6	6–7	6
		Lateral	0	0	0	0	1–4	1–5	2–4	2–4	5–7	5–8	5
		Ventral	0	0	0	0	1–4	2–6	2–7	3–5	5 +	5 +	6
III	Anterior	Dorsal	6–7	5–6	5	5	4	4	4	4	3	3	0
		Lateral	6	4–5	4–5	4	4	3	1–2	1	2–3	2–3	3
		Ventral	6–7	5–6	5–6	4–5	5	3–4	4–5	4–5	3	3–4	0
	Posterior	Dorsal	0	0	0	0	0	0	0–1	1	2–3	6–7	6
		Lateral	0	0	0	0	0	0	0	1	1	6–7	6
		Ventral	0	0	0–2	2–4	2–5	5	5–1	3–5	5–6	7	7–8

* *From four to five discontinuous rows plus two short continuous rows.*
** *Five discontinuous rows plus three short continuous rows.*

from the intermediate regions. The hooks are directed ventrally but less abruptly than in the first instar. They are toothless and much thickened throughout the anterior and intermediate areas. The two intermediate sections of the armature are irregular along the dorsal margins. The ventral margin of each contains two prominent protuberances. The posterior one is opposite the bridge between the two hooks. The ventral wings of the armature are moderately long. They are very dark at their base with a light central area and semitransparent blades. The dorsal wings are darkly pigmented except for a moderately pigmented fringe on the dorsoanterior, dorsal, and posterior margins. A semitransparent blade approximately 0.2 mm. long extends posteriorly from the pigmented border and is dorsad of the dorsal wing as a narrow margin.

Third Stage Larva

The third stage larva has an average length of 5.4 mm. with a range of 4.0 to 7.2 mm. The pseudocephalon is usually retracted into the first segment, and the posterior end of the body is more distinctly truncate than in the preceding stages. The 11th segment is moderately retracted and flattened. The larva is much grayer in color than in the preceding stages, and the cuticula is less transparent. Projections described by Hawboldt (3) as pseudopodia occur between the abdominal segments. A distinct lateral fold is present.

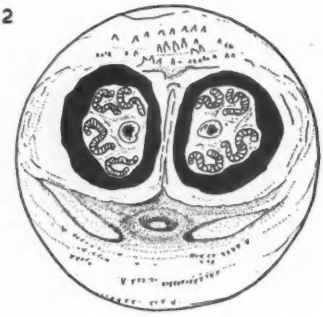
The armature of the cuticula consists of bands of spines which are flatter, fewer in number, and more regular than those of the second stage larva. The spines are unpigmented except for those surrounding the posterior spiracles. The arrangement of the spines is shown in Table I.

The larva is amphipneustic. The anterior spiracles (Fig. 10) project from the posterior border of the first body segment. They appear as short finger-like papillae with single orifices. The atrium of the felt chamber is elongate with a bell-like expansion at its base. The posterior spiracles (Fig. 11) resemble those found in the puparium and described later. The felt chambers are short and about as wide as long.

The pseudocephalon bears conspicuous preantennae and sensory organs as in the preceding stages and a few small spines directly below the buccal cavity.

The buccopharyngeal apparatus (Fig. 9) is well developed and may have two articulation points. The anterior and intermediate segments are closely associated but in some specimens are separated by a small fenestra. The anterior segments have each a broad ventral projection at their posterior margin, the anterior margin being smoothly toothed. The posterior dorsal margin has a broad raised portion. The salivary plate is small and inconspicuous. The intermediate segments have well-defined posterior margins. The medial ventral margins have each a large ventral projection, which is smoothly toothed on its anterior margin. A small sclerite is suspended in transparent tissue dorsad of the anterointermediate articulation. The posterior segments have each two well-defined wings, dorsal and ventral. The

12



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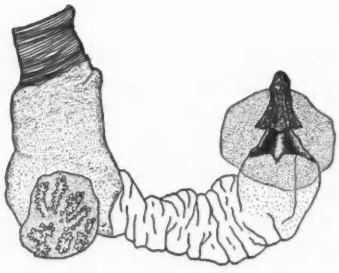

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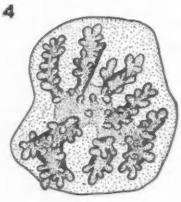

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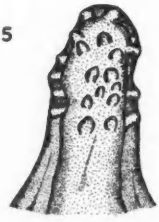

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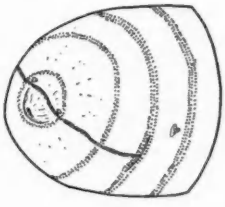

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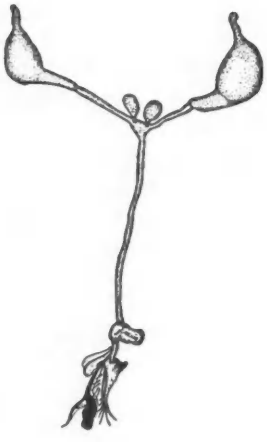

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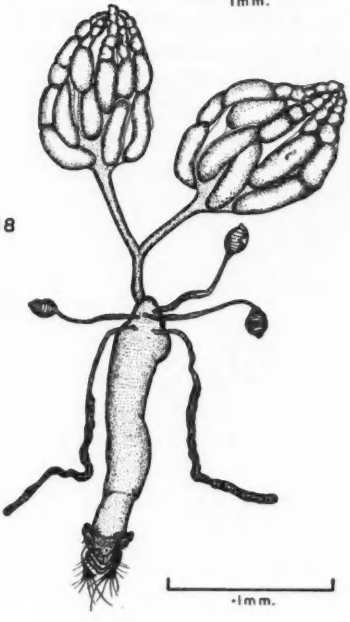

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darkly pigmented parts of the ventral wings are short, but the wings are continued for a short distance as lightly pigmented or as semitransparent blades. The two ventral wings together form a U-shaped trough. The dorsal wings widen abruptly; their anterodorsal margins project anteriorly as a hornlike structure. A small anterior area, a narrow dorsal, and a moderately narrow ventral margin are lightly pigmented. Translucent portions which merge into semitransparent blades project posteriorly from the lightly pigmented margins of the dorsal wings.

Puparium

The puparium varies from 4.5 to 6.3 mm. in length and from 2.0 to 3.0 mm. in width, the greatest diameter occurring one-third of the distance from the posterior end. Anteriorly, the puparium is rounded. It is subconical posteriorly and is usually slightly flattened ventrally. The puparium darkens with age and eventually becomes a rich mahogany or a very dark brown.

The posterior spiracles (Fig. 12) are not completely above the horizontal axis of the puparium and protrude slightly from a very shallow depression. They are roughly circular in outline, and shiny over the whole surface. They are very close together and flattened medially. Each spiracle contains four serpentine slits, which are bridged by thin, lightly pigmented bars. The button is moderately large and is near the mid-portion of the inside edge of the spiracle. Immediately below the spiracles is a prominent elevation with a deep median depression. This elevation is flanked on either side by a deep groove. An oval scar at the posterior margin of the tenth segment is all that remains of the anal opening. The two anterior spiracles (Fig. 16) appear as small conoid papillae and are slightly dorsad of the transverse axis of the puparium near the posterior margin of the prothoracic segment. The prothoracic spiracles (Fig. 13) are protuberant and are near the posterior margin of the fourth body segment. The prothoracic cornicles (Fig. 15) are well developed, each with at least 24 respiratory orifices. The internal spiracles (Fig. 14) contain 80 to 110 respiratory papillae arranged in radiating branches at the end of the atrium.

There are two lines of cleavage (Fig. 16) through which the two halves of the puparial cap are separated from the remainder of the puparium at the time of adult emergence. The horizontal line extends across the front, ventrad of the anterior spiracles and thence posteriorly to the anterior margin of the first abdominal segment. The vertical line of cleavage does not appear in its entirety but may be seen as a short crease on either side, extending for a short distance dorsad and ventrad of the junction with the horizontal line of cleavage.

FIGS. 12–18. *Phryxe pecosensis* (Tns.). 12. Central portion of posterior end of puparium showing spiracles, stigmatal openings, and prominent structures below. 13. Pupal respiratory apparatus. 14. Internal spiracle. 15. Tip of prothoracic cornicle. 16. Anterior end of puparium showing the two lines of cleavage, tips of anterior spiracles, and prothoracic cornicle. 17. Reproductive system of male. 18. Reproductive system of female.

Reproductive Systems

The structure of the female reproductive system (Fig. 18) follows the general form of tachinids that deposit macrotype eggs. Each of the two ovaries is made up of six to eight ovarioles, each of which produces at least six eggs. The lateral oviducts unite to form a common duct, which empties into the anterior portion of the uterus. The common oviduct is slightly more than one-half as long as the lateral oviducts. The three spermathecae are ovoid and darkly pigmented. Each spermatheca is covered with a varying number of concentric rings. On all the specimens prepared for study, each spermatheca bore a deep indentation situated approximately half of the distance from the base and extending across half of the circumference. The spermathecal ducts are slightly enlarged toward the uterus; the median duct enters the uterus just anterior to the entrance of the two lateral ducts. Immediately posterior to the spermathecal ducts are two accessory glands. These are slightly over twice as long as the lateral oviducts. The uterus is well supplied with a network of trachioles, which conduct air to the developing larvae.

The male reproductive organs (Fig. 17) are typically tachiniform, somewhat similar to those of *Aplomya caesar* (Ald.) as described by Wishart (9) and of *Phorocera hamata* A. and W. as described by Baldwin and Coppel (2). The paired testes are bulbular in shape, lightly pigmented, and joined to a common ejaculatory duct by a pair of moderately enlarged vasa deferentia. Attached to the upper portion of the ejaculatory duct are two, small, ovoid, unpigmented, and nearly sessile accessory glands. The ejaculatory duct is narrow and approximately three times as long as the vas deferens. Near the penis and appearing as a cylindrical enlargement of the ejaculatory duct is the ejaculatory pump.

Life History and Habits

Methods

For life history studies *P. pecosensis* adults were obtained from the spruce budworm received from British Columbia and handled in the manner described by Arthur and Coppel (1). Males and females were placed in separate cages. They were fed a 10% aqueous solution of honey and sprayed twice daily with tap water. Raisins were crushed and pinned on the inside of the cages to provide protein.

Observations on mating were obtained by placing one female with two males in a small cage (4 × 4 × 2½ in., outside measurements) for two to three hours daily. Since budworm larvae were not available for these studies, larvae of *P. rapae* were used as hosts. These larvae were placed in cages with mated females to obtain data on oviposition and preoviposition periods.

A record was kept on developmental progress of the parasites by daily dissections of host material. All rearing was carried out at a temperature of 23° C. and 60% relative humidity.

Life History

Mating was observed frequently in the laboratory. Females mated most readily when they were three days old. On one occasion a one-day-old female mated with a five-day-old male. In no instances were females observed in coitus after they were three days old and no females were observed to mate more than once. Males mated most readily two to seven days after emergence. Coition was completed in 30 to 50 min.

The preoviposition period varied from 7 to 14 days; the average time was 10 days. The oviposition period was relatively short, i.e., three to nine days.

The hatching time varied considerably. The first eggs deposited may either be infertile or take such a period to hatch that they become dislodged from the host's integument. Eggs deposited on the second and third days of the oviposition period hatched within six hours; those on the fifth day, within 20 to 30 min.; and those from the sixth to the eighth day, within 10 min.

The total time from egg deposition to the beginning of the formation of the puparium was 7 to 11 days, females taking longer than males. First stage larvae completed their development in two to four days. Three days were required in the second stage in all cases studied and from three to four days in the third stage.

The puparium was usually completed within a period of 24 hr. The adult emerged from 9 to 11 days after the formation of the puparium.

The life span of adults varied from 1 to 53 days with an average of 20 days.

Mating

Mating is usually accompanied by a brief courtship. While the female remains quietly stroking her prothoracic legs together or rubbing her metathoracic legs over her abdomen, the male walks rapidly toward her and then quickly retreats or flies to his original position. These advances may be repeated as often as six times, or until the male comes close enough to the female to touch one of her wings with his prothoracic leg. The latter act may be carried out from one to three times, and when the female shows no inclination to move, the male mounts her from behind. At the beginning of coition there are momentary adjustments in position, the female often attempting to dislodge the male by means of rapid backward thrusts of her metathoracic legs. The male overcomes this opposition by raising his metathoracic legs from the floor of the cage so that the female cannot touch him. The female becomes quiescent when the male is completely adjusted. The prothoracic legs of the male usually rest either on the tergal portion of the prothoracic region or at the bases of the female's wings. The claws of the mesothoracic legs of the male are hooked on the anterior margins of the wings of the female approximately one-third of the distance from the bases of the wings. Slight pulsations on the part of the male can be seen during coition. Upon separation each sex cleanses its genitalia with the metathoracic legs. Soon the male becomes very active and may fly in one or two circles above the female before leaving the vicinity. The female may either remain motionless for a time or walk slowly away.

Oviposition

In the laboratory, oviposition readily took place. Before oviposition, the female may remain facing the larva for some time. Then, with wings vibrating she approaches the larva at right angles to its length. She sometimes crawls over the larva several times without any attempt to deposit eggs. When deposition finally takes place, the posterior segments of the abdomen are extended and curved forward. The egg is deposited quickly, posterior end first, and usually attached at a fold in the integument with an adhesive substance. After an egg has been deposited, the fly walks away from the host and at right angles to it. Eggs are deposited anywhere on the body of the host but more generally on the lateral thoracic region. The greatest number of eggs obtained from a female in the laboratory was 32 and the smallest number was one. After depositing a few eggs, many females attempted to deposit more but were unable to do so; dissection showed that the uteri were congested with eggs containing well-formed first instar larvae. The reason for the unability to deposit them is unknown.

Hatching of Egg and Development of Immature Stages

The first eggs deposited may be infertile, and if so, they appear shinier and harder than those deposited later. The greater the intrauterine development, the shorter is the time required before eclosion. The larva cuts its way through the ventral surface of the chorion of the egg by means of its mouth hook. It then enters the host's body through the integument, leaving a slitlike scar with darkened edges. The abandoned chorion collapses, dries, and eventually drops from the host's integument.

Dissection of host larvae shows the first and second stage parasite free in the body cavity between the alimentary canal and the parietal fat layer of the thoracic region. Third stage larvae are found in the funnel-like structures attached to a host tracheae. The funnels are a milky-white color adjoining the tracheae and are delicate, almost transparent distally. The larvae are cradled in the funnels with their posterior spiracles closely adpressed to the tracheae. Upon completion of feeding, each parasite leaves the host larva or pupa to form its puparium.

When hosts are left for some time with a depositing female, several eggs may be deposited on a single larva. Dissections showed as many as five larvae feeding in one host but no more than three were ever observed to mature. Two larvae from a single host is not uncommon, and the adults produced are of moderate size. The largest and most robust parasites were obtained from host insects upon which single eggs were deposited.

Formation of the Puparium

The mature larva changes slowly to a cream shade when it emerges from the host. It becomes shorter and thicker. As the integument hardens, any deep wrinkles gradually smooth out. The puparial case is light yellow and semitransparent when first formed, but darkens to a rich mahogany within several days.

Sellers (6), quoting Regan, indicates that in the field, adults may be found from May until October. Two or more generations are encountered per year and the insects hibernate as larvae in the host.

Acknowledgments

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TEMPERATURE SELECTION IN DEER MICE[1]

By R. H. Stinson[2] and Kenneth C. Fisher[3]

Abstract

The behavior of individuals of the genus *Peromyscus* has been studied in an 8 ft. aluminum tube in which a gradient of temperature could be established. When the tube was at a uniform temperature the mice were found most commonly at the two ends. With the gradient set up providing a range of temperatures from 6° to 50° C. no mice were found at the hot end, the tendency to remain at the cold end was greatly reduced, and a broad peak in the distribution appeared between 20° and 30° C. This combined distribution suggests a much less precise selection of temperature than was characteristic of individual mice. The modes of the individual distributions varied from 20° to 28° C. thus accounting for the relative flatness of the combined distribution. The mode of the combined distribution was 24° C. A few experiments were done (1) with movement of air through the tube (2) in shorter gradients and (3) in a vertical gradient all of which point to the conclusion that it is the temperature of the substratum on which the animal walks that determines its response.

Introduction

It has been shown that many animals when placed in a gradient of temperature congregate within a certain restricted range, which is well within the lethal limits for survival. This behavior has been known for invertebrates since the time of Mendelssohn (18) and Jennings (15), the latter describing it in *Paramecium*. It occurs as well among the vertebrates, having been studied, for example, in fish by Doudoroff (6), Fry (8), Fisher and Elson (7); in amphibian tadpoles by Workman and Fisher (21); in reptiles by Lufti (17) and Herter (13); and in mammals by Herter (11, 12). Only birds among the vertebrates appear not to have been investigated in this connection.

A variety of terms have been used to refer to the behavior and to the temperature at which, or the range of temperature within which, the organisms aggregate. Such adjectives as "optimum", "preferred", or "selected" have been applied. The first of these has been used in connection with temperature for other reactions, e.g. rate of growth, and should probably be avoided unless it is qualified in some way, as Herter (11) does in his term "thermotactic optimum". The other two imply a somewhat anthropomorphic concept which is undesirable. It is convenient however to speak of the "selection" of temperature and of the "selected" temperature, and this usage will be followed here.

Temperature selection in mammals appears to have been studied first by Herter (11, 12). His gradient was set up in a metal bar and several rodents and bats were studied. He concluded that the organisms responded only to the temperature of the floor. This conclusion may be open to question

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to a degree, for the gradient used was small physically and an animal 4 cm. long could lie over a range of 1°–4° C. The values obtained were however confirmed in a similar apparatus by Bodenheimer (2) who investigated temperature selection in some rodents of Palestine. Kalabuchov (16) on the other hand examined two species of wild mice in a modified form of Herter's apparatus and obtained values much lower than those reported by Herter for the same two species. Similarly, lower values were obtained by Ponomarev (20) with larger mammals, actually several of the *Mustelidae*. Undoubtedly then, mammals commonly select temperature when in an environment nonuniform with respect to temperature. The exact values selected may be in doubt, but the data now at hand already suggest species differences and variations possibly ascribable to variations in the physiological state of the organisms.

It is evident from all this that in studies of the distribution and activity of small mammals in nature, the fact that they probably all select temperature will have to be kept in mind as one of the factors involved. It may turn out that under many circumstances the response to temperature will be subjugated to some other response. Nonetheless since temperature operates on the animal continuously, it is convenient to think of the response to temperature as a framework of behavior which, because it is surrounded by other possible responses that are frequently given precedence by the central nervous system, may only occasionally or at special times emerge in a dominant role.

Of all the small wild mammals found in North America, none probably is more common than the genus *Peromyscus*. Being numerous it is an important element of food for larger forms. As an example to be used in studies of distribution and activity, it appears quite typical and, as a consequence, a variety of data pertaining to this genus is accumulating (see for example, Behney (1), Clark (4), Dice (5), Hart (9), Hatfield (10), and Moody (19)). *Peromyscus* is, in addition, easily maintained in the laboratory. It was considered of importance to study the reactions of this organism in a gradient of temperature since this has not been done thus far. The present paper records such an investigation.

Materials and Methods

The organisms used throughout most of this investigation were members of the first and second generations of a stock of *Peromyscus maniculatus bairdii*, Hoy and Kennicott, obtained from the sand beach at Long Point, Lake Erie. The colony was kept in a room subject to the normal daily fluctuation of light intensity and without control of temperature or humidity. Intermittent recordings indicated that the daily variations of temperature amounted to as much as 8° C. (i.e. from 15° to 23° C.) in winter and occasionally to as much as 12° C. (i.e. 21° to 33° C.) in summer. Throughout the experiments the animals were fed on a standard diet of "Master" fox chow.

The horizontal temperature gradient to which the organisms were exposed was set up along the length of an aluminum alloy tube 10½ ft. long with an inside diameter of 3⅜ in. and a wall ½ in. in thickness. To permit observation

of the animal in the tube, a longitudinal slot was cut out of the wall from a point 1 ft. from one end to a similar point 1 ft. from the other end. The sides of this slot were perpendicular to the outer surface of the tube and at that surface the slot was 1⅝ in. wide. A lucite window ½ in. thick was cut to fit tightly into the slot and was sealed in place with cellulose tape. To facilitate recording the positions of the animals, crayon pencil lines were drawn across the window at intervals of 2 in.

One end of the tube was sealed, for a distance of 12 in., into a copper tank which was a 1 ft. cube. A mixture of crushed ice and salt, put into this tank directly in contact with the surface of the tube, cooled this end. The other end was heated in a similar copper tank containing a brass sleeve which fitted closely about the tube and allowed the tank to be moved along it. The water in this tank was heated to 60° C. and was constant within $\pm 0.25°$. Except for the window, that part of the tube between the tanks was covered with a 1 in. layer of felt insulation held on by wires passing around the tube. The gradient of temperature in the tube was found to be stable two hours after the application of the heat and cold respectively. Only then were experiments begun.

The variation of temperature along the tube was almost exactly linear so that equal intervals of temperature represented equal distances. The length of the gradient available to an animal was determined by the positions of two movable barriers (glass bottles fitting the tube closely). To change the length of the gradient, and hence its steepness, both the barrier and the heating tank at the warm end were moved to the required position. To lessen air currents through the tube, a tight-fitting fiberboard plug was inserted into each end. The interior was illuminated by the light from a bank of ten 40-watt lamps arranged in front of and below the tube in such a way that they could not be seen by an animal within. The light from these was reflected down into the tube from a strip of white paper suspended above the window. In front of the lights was a barrier of plywood which extended upwards to within 1–2 in. of the edge of the white paper reflector. Observation was carried on through the slit thus formed. There was no general illumination in the room in which these experiments were done.

Temperature was measured with copper–constantan thermocouples made of No. 30 B. and S. gauge wire. They were inserted between the tube and the felt insulation in such a way as to indicate the temperature of the part of the tube on which the feet of the mice normally rested. To obtain the temperature of the air in the tube the thermocouples were inserted as desired through small notches cut in the edge of the lucite window. Since for practical purposes temperature was a linear function of distance along the tube, the temperature of any position between those at which thermocouples were inserted was easily obtained by interpolation.

During preliminary phases of the investigation it was observed that if several animals were present in the tube at the same time, they tended to huddle together or lie on top of one another in such a way that it was no longer possible

to know to what temperatures they were exposed. The data to be reported here, therefore, were obtained with only one animal at a time in the tube. It was recognized that the tube itself might have an effect on the behavior of the organism. To avoid confusion from this, it was necessary to judge the effect of the gradient by comparing the behavior in the tube with and without the gradient established in it. When the two types of experiment were being done, the one with the gradient, the other at constant temperature, they were done alternately so that the same temperature conditions were not present in consecutive experiments. This procedure made it less likely that comparisons of the behavior under the two circumstances would be affected by any possible modification of behavior produced either by previous experience in the tube (a "serial" effect) or by the presence of any preceding organism in the tube.

The animal to be used was taken from the colony without any special preparation and was usually inserted into the tube from the cold end, the gradient, if used, having already been established as described above. Since the mouse typically exhibited considerable restlessness for a period of half an hour or more, no readings were taken until after one hour. At this point floor temperatures were taken and the recording of the position of the mouse was begun. Specifically it was the mid-point of the ventral abdomen which was used as a reference point and the location of this spot was noted once each minute along with the direction in which the animal was facing. It was much more common for the longitudinal axes of the animal and tube to be parallel than for them to be at right angles to one another, although the latter occasionally did occur.

The readings of the position of the animal were continued for one hour. The temperature of the floor was measured at the beginning of the observation period, as indicated above, again 30 min. later, and finally at the end of the observation period. If an animal remained in any one position for five minutes, an attempt was made to disturb it. This was considered necessary in order that the animal might experience the different temperatures along the gradient. Usually all that was necessary was a movement of one of the end barriers. If repeated disturbances had to be made during an experiment, they were made at alternate ends of the tube.

After each experiment the tube was cleaned by pushing a tight-fitting dampened cloth through it from the hot end.

Results

It is appropriate to consider first the observations made without the gradient of temperature set up. Animals in the tube with the latter at a uniform temperature of approximately 23° C. were found to be either (1) running back and forth from one end to the other, (2) attacking the end barriers by gnawing and scratching at their edges, (3) grooming, or (4) completely inactive.

Attacks on the barriers kept the animals at the ends of the tube. So also did grooming, for this frequently followed an attack on a barrier or occurred at the end of a period of running which was terminated upon arrival at a barrier. Furthermore when an animal came to rest, it usually did so with the body pressed into the angle formed by the barrier and the floor. It thus happened that three of the four types of behavior which could be readily distinguished tended to increase the time spent by the animals at the ends of the tube. The consequence is well illustrated in Fig. 1*A* which is the record of the positions at which 15 animals were observed during experiments in which the temperature remained the same along the length of the tube. It is evident that the ends were very strongly selected while in the remainder of the tube the distribution was fairly even.

With the gradient set up, the behavior in the tube was somewhat different from that seen without the gradient. The animal always moved away very quickly from the hot end and attacks on the barrier at this position completely stopped though they still occurred at the cold end. Both grooming and complete inactivity were seen much less frequently at the ends, and in fact almost never at the hot end. They appeared instead over a region in the central part of the tube. The resultant distribution of the same 15 animals referred to above is shown in Fig. 1*B*. The peak at Position 48 has disappeared and that at Position 1, though still present, has been considerably reduced. Midway along the tube a broad hump has appeared. It is evident that the existence of the gradient has greatly modified the distribution of the animals in the tube. Inasmuch as the activities of the animals tend under these circumstances to be confined to a restricted range of temperature, the animals may be said to have "selected" temperature.

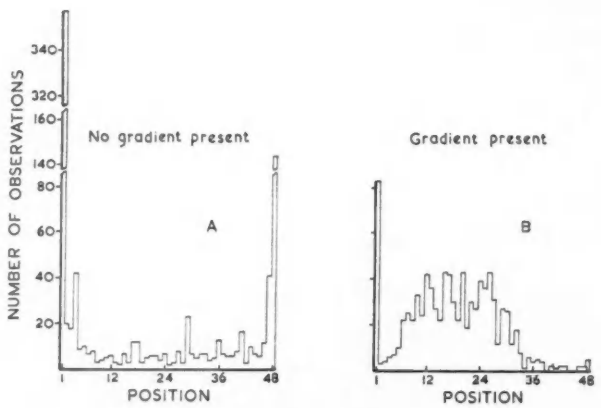

FIG. 1. *A*. The frequency with which *Peromyscus* was observed at the different positions in an aluminum tube at constant temperature. Combined data on 10 individuals. *B*. The frequency with which the same animals were observed when a temperature gradient extending from 6° to 50° C. was set up in the tube. Position 48 indicates the hot end.

From the description of the behavior in the gradient, it is evident that it was the change in the positions of grooming and resting which largely accounted for the appearance of the peak of selected temperature and for the reduction of the peaks at the cold and at the hot ends. With a particularly active animal which seldom came to rest, the region of selection was defined largely by grooming behavior.

It is of interest that the position of the body of the animal when at rest in the cold end often differed from its position in the central region of the tube. On the cold floor the body was hunched, the feet close together, and the abdomen quite possibly raised from the floor, though this was not determined with certainty. It appeared too that the head was held off the surface of the floor at the cold end. In the central region, on the other hand, the hunched position was not evident. Instead an animal here often appeared to flatten itself against the floor, with the head seemingly resting on the surface.

In such an apparatus as that used for these experiments, there is present not only a horizontal gradient in the floor of the tube but also both a horizontal and a vertical gradient in the air above the floor. In order to specify the exact range of temperature selected by the animal, it is clearly necessary to know to which of these gradients or to what combination of them, the animal responded. Information pertinent to this point was obtained from experiments in which the air temperature was changed without change in the floor temperatures. The procedure was to determine the distribution of the animal in the gradient in the usual way; air at room temperature was then passed through the tube from the cold end at the rate of three liters a minute and the distribution was again determined. To limit the total duration of the animal's stay in the tube to the usual two hours, each of the observational periods was made only 30 min., the first being without the air movement, the second with it.

The current of air had quite different effects on the floor and air temperatures along the tube. At most positions the floor temperature, if it changed at all, did so usually by less than one degree. The air temperature on the other hand, over regions where the peak indicating selection occurred, changed by as much as eight degrees in the 6 ft. gradient where the greatest differences were observed. Nevertheless it was not possible in the three experiments done to detect any difference in the positions at which the animals were found owing to the presence or absence of the current of air. It was concluded that the animals were selecting according to the temperature of the floor.

It became apparent that an individual might exhibit some variation in the temperatures selected in different experiments. The extent of this is indicated by the four distributions obtained during the summer months for animal No. 38 which are shown in Fig. 2. It may be seen that the mode, which is usually taken as *the* temperature selected, varied from 21° to 29° C.

To obtain a value for the selected temperature which might be somewhat characteristic of the species, having in mind this variability between individual

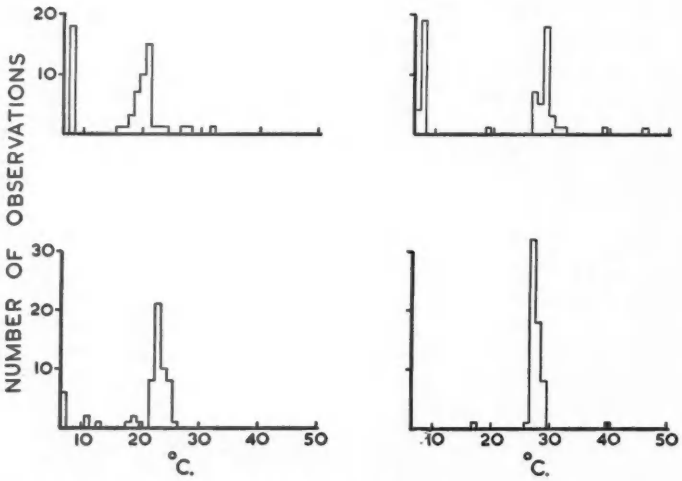

FIG. 2. The frequency with which an individual *Peromyscus* was observed at the various temperatures of a gradient. The data from four separate experiments on the same animal are shown.

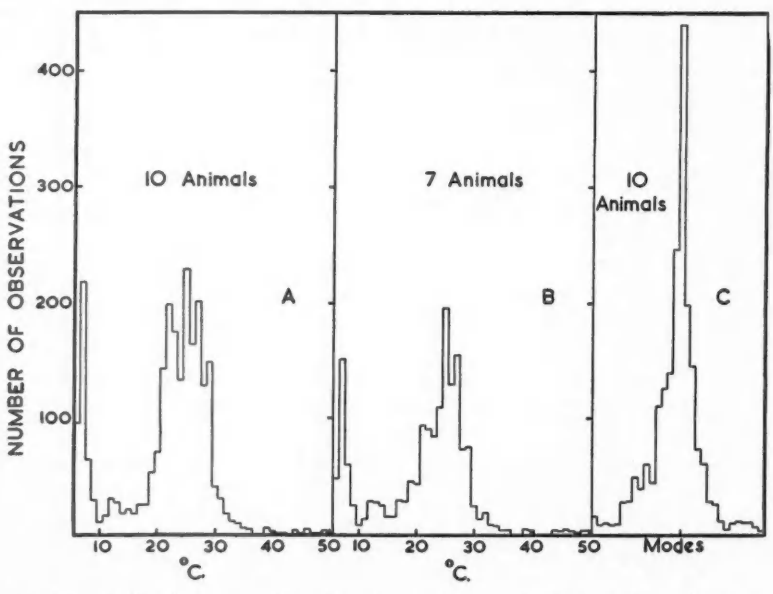

FIG. 3. *A*. The frequency with which *Peromyscus* was observed at the various temperatures of a gradient. These are the combined data from 10 animals each of which was observed in the gradient upon four occasions. *B*. The data of *A* with the omission of the observations from the three individuals which differed most from the mean behavior. *C*. The data of *A* with the temperature axes for the different mice shifted so as to make the 10 modes coincide.

experiments, four distributions were taken for each of 10 animals. All of these were recorded during the spring and summer months. The distribution of the 2400 observations which resulted is shown in Fig. 3*A*. It is evident that a broad peak appeared over the region between 20° and 30°, and a sharp one at a temperature of 6°–7°. These latter temperatures existed only at the end of the tube and since a similar peak at the end occurred without the gradient set up, it is evidently not characteristic of temperature selection. It will be noted that there is no peak at the hot end although one did occur at this position, as was noted earlier, when the tube was at a uniform temperature. The disappearance of this peak when the gradient was used is undoubtedly due to the presence of high temperatures at this position.

The peak in the distribution of observations which fell in the region 20°–30° C. is the evidence from which it was concluded that these animals selected temperature. Its modal value is 25° C. The distribution may be further characterized by its mean, which is 24.1°, and its standard deviation which is 4.3° C. In calculating these last two values only that part of the distribution lying between 12° and 36° was used, since the accumulation at the cold end was not produced by temperature.

In Fig. 3*A* it is evident that within the broader peak smaller peaks appear. An examination of the individual distributions revealed the fact that these smaller peaks were due to certain animals. If for example Fig. 3*A* is replotted omitting the appropriate three animals, the distribution shown in Fig. 3*B* is obtained. The subsidiary peak at 22–23° in Fig. 3*A* to which two of the three contributed heavily has now been greatly reduced, and that at 29° due largely to the third animal has almost disappeared. The spread of the distribution in the temperature gradient is thus due not only to variation within an individual, as demonstrated, but also to variation between animals.

From a theoretical standpoint it is of interest to know the precision with which temperature is selected on the average in any one animal. An indication of this was obtained by taking from the distributions of each of the 10 animals used to assemble Fig. 3*A*, that part which fell in the range 12–36° and then shifting these distributions along the abscissa until the individual modes coincided. The result is given in Fig. 3*C*. The modal interval now contains 22% of the observations while in Fig. 3*A* it contains only 12%. Grossly this new distribution suggests a more precise selection than that suggested by Fig. 3*B*. The tendency of a distribution such as this to peak may be measured quantitatively by its kurtosis. This is a statistic which measures the amount by which the distribution in question departs from a normal distribution. The appropriate calculations (Chambers (3)) show that the distribution in Fig. 3*C* is very significantly leptokurtic, i.e. very much more peaked than is a normal distribution. The distribution in Fig. 3*B* on the other hand does not differ significantly (5% level) from a normal distribution. It follows that the normality in this latter case cannot be taken as a characteristic of the

temperature selection process. Instead it probably indicates that the modes of the individual mice were normally distributed about a mean value. It may be of interest to record here that the respective modes for the 10 animals involved in Fig. 3 were, to the nearest degree, 20°, 22°, 22°, 25°, 25°, 25°, 26°, 27°, 27°, and 28° C.

Selection in Shorter Gradients and in a Vertical Gradient

The selected temperatures recorded for several species of small mammals by Herter (12) and by Bodenheimer (2) are in general several degrees higher than those reported here for *Peromyscus*. In addition Herter (12) and Kalabuchov (16) using similar species but a shorter, and hence steeper, gradient reported modal temperatures which are higher than those reported here by as much as 13° C. It is not difficult to believe that these differences in selected temperature all represent differences between species and even between strains of the same species. It is also possible of course that the organisms used in these several investigations were not similarly acclimated to temperature, and even conceivable that the actual temperature selected, as reported, depended to a degree on some characteristic of the apparatus in which it was determined. It seemed useful therefore to make a few measurements in gradients steeper than those used for the observations reported above, and also in a vertical gradient.

Accordingly several experiments were done in which the length of the tube available to the animals was decreased, at first to about 6 and then to 4 ft. by moving the barrier at Position 48 (8 ft. gradient) to either Position 35 (6 ft. gradient) or Position 24 (4 ft. gradient). At the same time, by moving the heating tank along the tube, it was possible to keep the same temperature range over all three lengths. The gradient of temperature thus became steeper as the length of the tube decreased.

The general behavior of the animals in the shorter lengths was much the same as described for the 8 ft. length. Again without the gradient the peaks occurred at the ends, whereas with the gradient present that at the cold end was reduced and that at the hot end usually disappeared. No significant difference was found between the distributions of five animals obtained in the 6 ft. and 8 ft. gradients, and between the distributions of four animals obtained in the 4 ft. and 8 ft. gradients. It appears that shortening the length of the gradient had no significant effect on selection.

As a further check on the apparatus and method a few observations were made on the laboratory-reared albino white mouse, *Mus musculus* L. for this organism has been the object of at least two other investigations. Herter (12) has reported a mean temperature of 34.6° C. for the distribution of this animal in a gradient, the lowest value found for an individual mouse being 33.9° and the highest 35.6° C. The mean value observed by Bodenheimer (2) for the same species was 31.4° C.

For the present purpose only three of these animals, two males and one female, all of unknown stock were used. The modes of the distribution of

these individuals in a 6 ft. gradient were respectively 32°, 33°, and 36°. One of the three was also studied in an 8 ft. gradient, the mode of the distribution in it being 35° C. The means of these four distributions, to the nearest degree were, respectively, 31°, 33°, 34°, and 34° C. These values thus fall in exactly the same range as those obtained by Herter and Bodenheimer for the same species but using an apparatus of much different construction. It is concluded that the differences between our observations on the prairie deer mouse and those reported by Herter and by Bodenheimer for related species cannot be accounted for by procedural or instrumental variations. Instead they must be ascribed to real species and strain differences, or possibly to differences in acclimation.

Many authors have commented upon the vertical gradients of temperature which exist in the air above the metal floor in such an apparatus as that used in the experiments described above. Actually the tube used in the present investigation is a little less subject to such vertical gradients than are equipments in which the metal used in the construction is confined to the bottom or sides of a trough. However there is still an appreciable vertical gradient even in the tube, and as pointed out earlier it is necessary in the interest of precision to establish whether it is air temperature or floor temperature which is determining the distribution. One experimental approach which relates to this question has already been described. Another involves the use of a vertical gradient instead of a horizontal one and it will now be considered.

In Fig. 4*A* is shown a 4 ft. high wooden structure consisting of six floors and interconnecting ramps. It fitted snugly into a watertight metal container

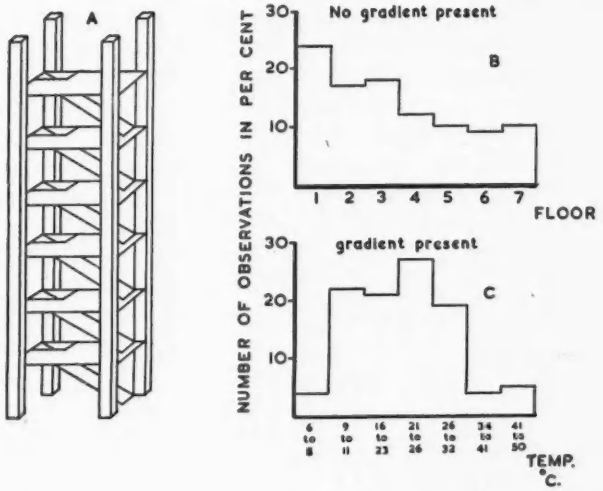

FIG. 4. *A*. The arrangement of interconnecting ramps used in the vertical gradient. *B*. The frequency with which *Peromyscus* was observed on each "floor" in the vertical "gradient" before the gradient of temperature was established. *C*. The same as *B* after establishing the temperature gradient.

with a plate glass window. The bottom of the container was cooled by immersing it to a depth of 6 in. in a bath of water and ice. Heat was applied to the top by suspending over it a wooden box with heating coils on the inner surface of three of its sides. The fourth side contained a window through which one could observe the interior of the "apartment house". This arrangement produced a vertical gradient of temperature in which the behavior of four specimens of *Peromyscus leucopus noveboracensis* (tentative identification) were studied. Temperatures were measured at a distance of 1 in. above the center of the bottom (floor 1) and each of the other six floors by inserting thermocouples through glass tubes in the sides of the metal container. This was done at the beginning and again at the end of an experiment by which time the temperature on each floor had usually shifted upward by one degree.

One mouse was used at a time and it was left in the gradient about one hour before observations were begun. Then the positions were recorded at one or two minute intervals for a period of one to one and a half hours. If an animal remained on the same floor for five consecutive observations, it was disturbed by a blast of air through the glass tube. The experiments were carried out in a room illuminated by both daylight and artificial light, and no attempt was made to control the light intensity inside the apparatus.

The general behavior of these animals in this apparatus resembled that of *P.m. bairdii* in the horizontal gradient. Running activity often consisted in moving quickly up and down the ramps. Resting and grooming were again evident. When the animals came to rest here, they often did so with the body pressed into the angle formed by the glass window and metal wall of the tank.

Fig. 4*B* shows the combined distribution of the four animals without the gradient set up and Fig. 4*C* the distribution with the gradient set up. In the latter case the temperatures are given in ranges because the same temperature was not present on each floor in each experiment. During any one experiment as mentioned above, the temperature on a given floor did not usually vary by more than one degree. In this structure, as with the horizontal arrangement, uniform temperature conditions, as when no gradient was established, did not lead to a uniform distribution of observations. In this instance (Fig. 4*B*) the greatest number of observations occurred at the bottom and the number gradually decreased towards the top.

It may be seen from Fig. 4*C* that the relative number of observations at the top and bottom, particularly the latter, became less when the gradient was established, with the result that a broad centrally located peak appeared in the distribution. The modal value of the temperature occurs in the 21° to 26° C. interval although it is clear that selection by this species in this equipment was not at all sharp. Earlier it was concluded that it was the floor temperature to which the mouse responded. This being the case it can be surmised that the relatively sharper selection on a metal floor is the result of the relatively higher thermal conductivity of the metal floor.

Acclimation

It has been reported by Herter and Sgonina (14) that the temperature selected by rodents is affected by the environmental temperature to which they have been subjected prior to the selection experiments. In other words the temperature selected is subject to an acclimation to temperature. In experiments with house mice these investigators found that the selected temperature dropped significantly as a result of exposure to high temperatures and rose after exposure to low temperatures. The direction of the change in the selected temperature with the acclimating temperature here is the reverse of that found in certain cold blooded forms (Doudoroff (6), Workman and Fisher (21), Fry (8)). A few experiments were made using the prairie deer mouse to determine the direction of the change in this case. The distribution given by four animals which had been exposed to room temperatures (22–23° C.) was determined. These animals were then moved to an environmental temperature of 33° C. and after two to five weeks were again studied in the gradient. Finally they were studied for the third time after an exposure to 14–15° C. for two to five weeks. The modal temperatures of the distributions obtained after each exposure are shown in Table I.

TABLE I

MODAL TEMPERATURES OF THE DISTRIBUTION OF FOUR DEER MICE IN A GRADIENT OF TEMPERATURE AFTER EXPOSURE TO THREE DIFFERENT ENVIRONMENTAL TEMPERATURES

Animal No.	Temperature of environment		
	22° C.	33° C.	14–15° C.
38	31°	23°	29°
63	26°	22°	23°
65	24°	21°	25°
57	25°	24°	25°

It will be noted that after raising the environmental temperature to 33° C. the mode in each case fell; while after lowering it from 33° to 15° the mode in each case rose. It is true that the amount of change was only one degree in two cases. The direction of the change was the same in all animals however. It appears then that exposure to high temperatures leads to a lowering of the temperature selected and vice versa.

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EFFECTS OF SALINITY ON THE DEVELOPMENT OF THE PACIFIC HERRING[1]

By R. G. McMynn[2] and W. S. Hoar[3]

Abstract

Successful methods have been developed for the artificial fertilization and rearing of the eggs and larvae of Pacific herring, *Clupea pallasii* Cuvier. A salinity optimum (11.53‰ to 16.24‰) for development and survival of eggs and larvae was evident in a study of the effects of constant and changing salinity (0‰ to 34.28‰). However, there is a wide salinity tolerance (6.06‰ to 34.28‰) for both eggs and larvae. Eggs transferred to pond water (0‰ salinity) during the first few days of development perish within a few hours, but if transferred at a later stage, they will survive for at least two days. No apparent correlation exists between survival of eggs and magnitude of salinity change. During development, two critical stages are apparent: the first at blastopore closure, the second prior to and during hatching.

Introduction

Salinity is an environmental condition which remains remarkably constant in the open ocean but can vary considerably in coastal waters. As herring spawn inshore, often near estuaries, eggs and larvae must encounter rather variable salinities. In the spring of 1949, on one of the larger spawning grounds of the North Pacific (Queen Cove, Esperanza Inlet), eggs were deposited in areas over which a considerable range of salinity was recorded (8.21‰ to 27.68‰). Limited observation, in this case, indicated that these eggs seemed to develop and hatch normally. However, it is possible that salinity might, in certain localities, be a limiting factor in the survival of eggs and larvae.

The following experiments were therefore undertaken in an attempt to show the effects of some constant and some varying salinities on the survival and development of the Pacific herring, *Clupea pallasii*.

Methods

The experiments were carried out at the University of British Columbia on eggs of herring captured in Departure Bay, through cooperation of the Pacific Biological Station of the Fisheries Research Board of Canada. Preliminary experiments in which live fish, dead fish, and recently fertilized eggs were transported from Departure Bay showed that the first procedure (Experiment 3) gave best results (Fig. 1).

In the laboratory, eggs were stripped from ripe females onto one side of a microscope slide (75 × 25 mm.) to form rows with eggs one or two layers thick. These egg slides were placed in a container of sea water of the desired temperature and salinity. Sperm were than stripped into these containers. The egg slides remained in sperm suspension for about 10 minutes after which

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Contribution from the Department of Zoology, University of British Columbia, Vancouver, B.C., with financial assistance from British Columbia Packers, Limited.
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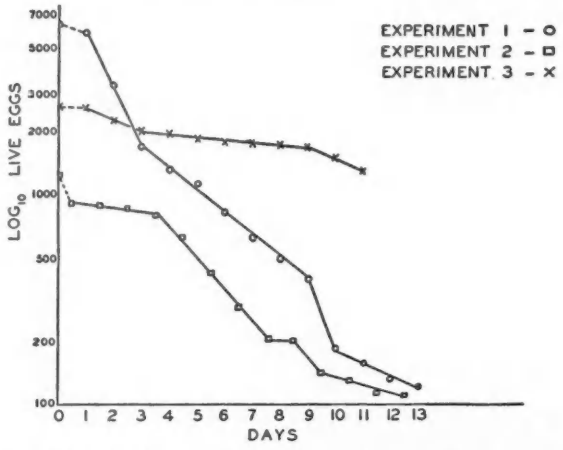

FIG. 1. Survival of herring eggs. Experiment 1, eggs stripped onto glass slides, fertilized at Departure Bay, and immediately transported to University (five hour journey at 7.5° C.–8.5° C.); Experiment 2, eggs taken and fertilized 16½ hours after death of fish during which time fish were shipped to University; Experiment 3, eggs and sperm procured from live fish shipped from Departure Bay to University.

they were transferred, unwashed, to a series of 600 ml. rearing beakers suspended by a wooden frame in a constant temperature bath (8.5 ± 0.5° C.). The incubation temperature was arbitrarily chosen and based on the observation that temperatures varied from 5.8° to 10.0° C. on herring spawning grounds. The sea water in the beakers was aerated continuously. Observations were made by removing slides from rearing beakers, placing them in Petri dishes containing sea water of the same temperature and salinity and observing with a binocular microscope.

A series of rearing solutions varying between 0‰ and 34.28‰ salinity were prepared in 48-liter glass containers. These solutions were made up by the addition of either sea-salt or pond water to English Bay sea water of known salinity. The 0 ‰ salinity solution was pond water taken from a small fish pond on the University campus. Precise salinity values in parts per thousand for the eight solutions used were: 0, 6.06, 11.55, 16.24, 22.30, 26.13, 30.26, and 34.28.

Results

General Description of Early Development

External changes occurring in Pacific herring from fertilization of the eggs to hatching (8.5° C. and a salinity of 27.43‰) are illustrated in Figs. 2 and 3. Eight hours after fertilization the first cleavage plane becomes apparent and by the end of the first day gastrulation has commenced. Neuralation commences around 20 hr. and is completed before 50 hr.—corresponding to the closure of the blastopore. Towards the end of the second day, primordia of the eyes (the optic vesicles) are seen. During the latter part of the third

day, epiboly has proceeded to such an extent that the yolk mass has been completely overgrown by ectoderm. Unpigmented eyes are prominent in the four-day-old embryo. At the end of the fourth day, the embryo has elongated so that it extends about three-quarters of the way around the yolk sac. During the fifth day of development body somites and pectoral fins make their appearance. On the fifth or sixth day, the body of the embryo begins to twitch

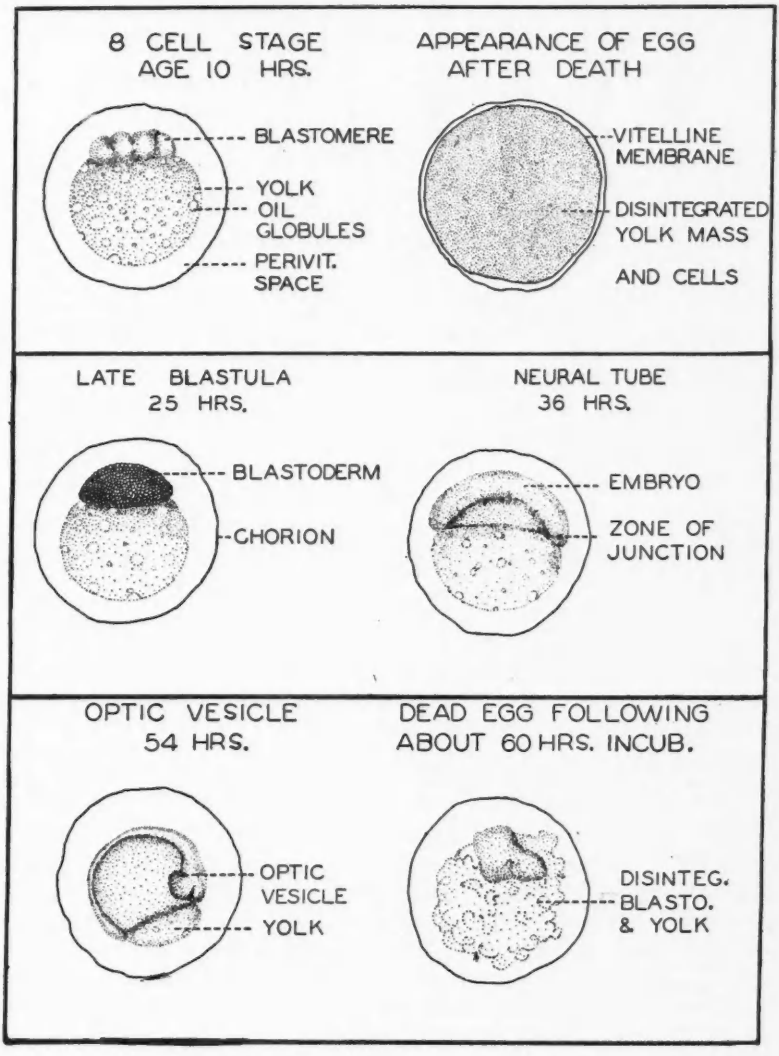

FIG. 2. External appearance of herring embryos during the first 54 hr. of incubation (8.5° C., and 27.43‰ salinity)

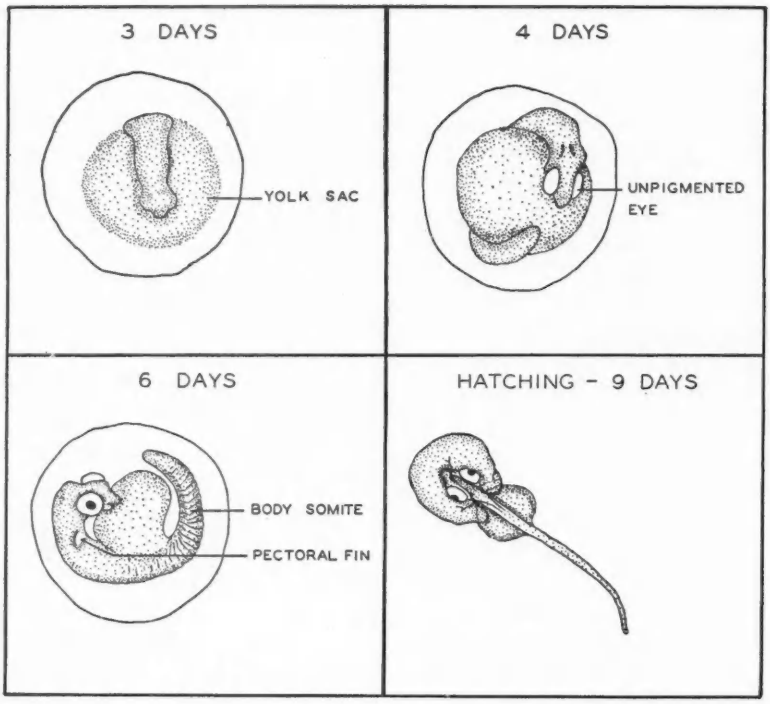

FIG. 3. External changes in herring embryos from the third day of incubation until hatching (8.5° C. and 27.43‰ salinity).

spasmodically. The ninth day is probably best characterized by the commencement of a violent thrashing about of the embryo, especially the tail region. Hatching commences during the latter part of the 9th or 10th day. All living eggs had hatched by the 12th day.

Larvae were successfully cultured beyond the yolk sac absorption stage on nauplii of the brine shrimp, *Artemia salina*. The literature indicates that previous rearing experiments have met with little success (9). The relative success of three diets used in these experiments is shown in Fig. 4. A proper kind of living food is apparently essential. In these experiments herring larvae, eight days after hatching, were observed actively pursuing brine shrimp larvae. In many of the herring at this time, nauplii were plainly visible in the intestine. Active feeding continued for 20 days when 56% of the larvae were still alive. In 20 days the herring larvae had grown from a hatching size of 6.0 mm. to 10.2 mm.(mean total length).

The general appearance of a recently hatched larva and a larva about two weeks old are shown in Fig. 5.

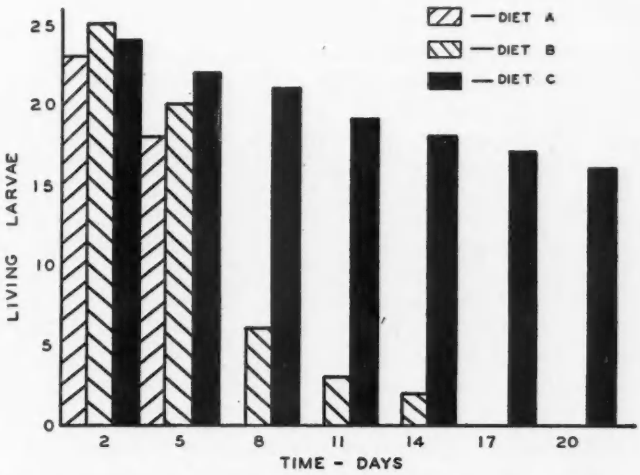

FIG. 4. Survival of herring larvae on diet A, *Daphnia pulex* eggs; diet B, liver – skim milk; and diet C, *Artemia salina* nauplii.

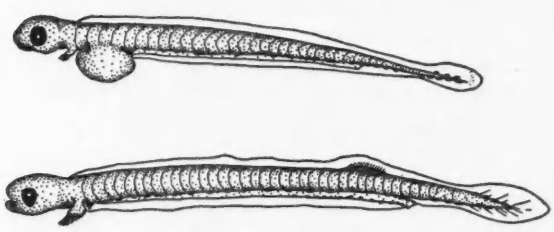

FIG. 5. Herring larvae; upper, recently hatched; lower, two weeks old.

Constant Salinity Experiments

For each of the eight different salinities, eggs from one female were stripped onto eight glass slides and fertilized in the milt solution for approximately 10 minutes. Immediately following fertilization the egg slides were transferred, unwashed, to four 600 cc. beakers, two slides to each beaker. These beakers were immersed in the constant temperature bath. Water in the beakers was aerated continuously and changed every other day. The above procedure was carried out for each test solution; thus the whole experiment consisted of 32 beakers each containing two egg slides, or four replications for each of the eight salinities. Observations on development and mortality were made by examining each slide every second day. Total numbers of eggs involved and survivals (expressed as percentages) are shown in Table I.

During the experiment there appeared to be a differential mortality among the egg slides held in the *same* salinity. For example, six of the eight slides in one of the salinities at a particular stage of development might show a survival

TABLE I

PERCENTAGE SURVIVAL AT VARIOUS AGES OF EGGS FERTILIZED AND HELD IN CONSTANT SALINITIES

Salinity, ‰	Original no. eggs	Age in hours					% Eggs producing live larvae
		16	61	106	157	204	
		% Survival					
0	5446	0	0	0	0	0	0
6.06	2783	77	48	44	37	30	0.2
11.55	876	88	80	75	74	63	8.3
16.24	894	84	56	50	45	40	9.4
22.30	1185	87	79	67	62	37	1.1
26.13	1065	82	38	36	28	16	0.8
30.26	1086	80	77	61	32	17	0.4
34.28	1258	62	58	52	40	36	11.8

of about 70%, whereas the remaining two slides, in a different beaker, showed a 20% survival. Fig. 6 represents the frequency of occurrence of per cent survival groups during the last three observations before hatching commenced. There seems to be a more or less well defined trend which indicates a high frequency of high and low mortalities with a relatively low frequency of occurrence of the intermediate survivals. This would seem to indicate that there is a high percentage survival on egg slides until some factor, perhaps the production of toxic substances from dead eggs, appears and quickly kills

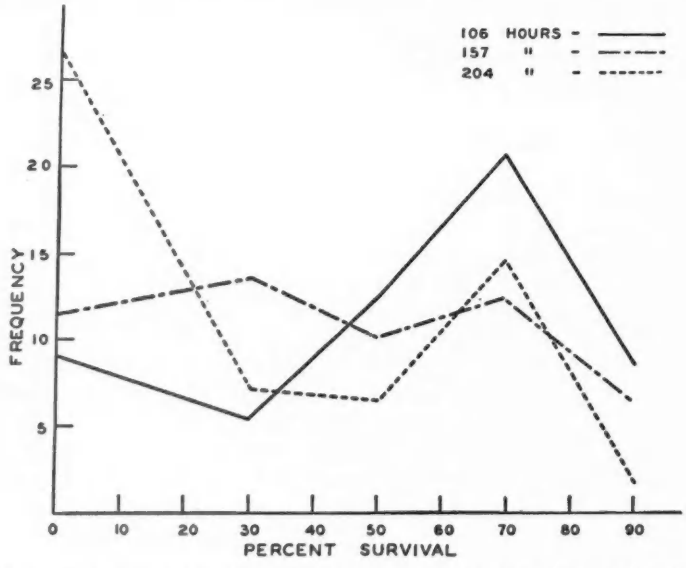

FIG. 6. Frequency of occurrence of percentage survival groups of eggs during the last three observations prior to hatching (further description in text).

practically all of the eggs on the slides in a particular beaker. In the analysis of the data, slides exhibiting this apparently excessive mortality were treated along with the rest of the egg slides. Perhaps smoother trends would have resulted if these slides had been ignored.

Survival data (percentage) were tested for significance by the multiple classification treatment given by Snedecor (11, pp. 275–280). Details of calculation are given in the thesis from which these data are taken (7). The analysis indicates that: (*a*) interaction is not significant, i.e. various constant salinities do not have different effects at any particular age; (*b*) there is a significant difference ($P < 0.01$) in the per cent survival as age increases; and (*c*) survivals which differ significantly ($P < 0.01$) result from the application of various salinities.

Fig. 7 illustrates percentage survival in various salinities at the indicated ages. There is no survival in the 0‰ solution, and although there is a significant difference in survival in the other salinities it is apparent that there is a

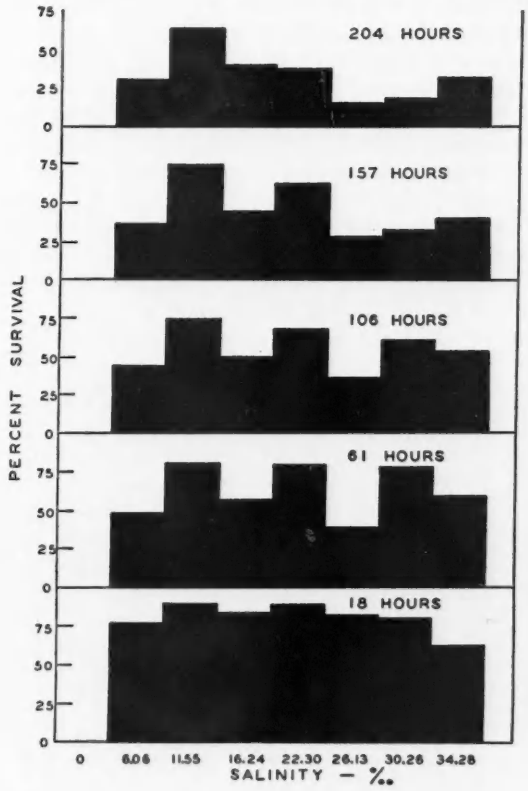

FIG. 7. Percentage survival (of original numbers of eggs) at different ages in various salinities.

TABLE II

MORTALITY INCREMENTS (%) CONSTANT SALINITY EXPERIMENT

Age in hours	Salinity (‰)						
	6.06	11.55	16.24	22.30	26.13	30.26	34.28
0	17	6	12	9	9	10	9
18	6	4	6	3	11	10	23
61	27	10	26	10	52	4	7
106	10	7	19	15	8	19	9
157	14	1	10	15	14	48	14
204	22	16	11	38	40	44	16
240	23	4	21	42	64	46	14

wide range of tolerance. A marked irregularity is obvious during the first 61 hr. of incubation, but subsequently a relatively higher survival occurs in the 11.55 ‰ salinity. There seems to be a general trend with rapid increase in egg survival from 0‰ to 11.55‰. A more or less gradual decrease in survival to 30.26‰ follows at which point a marked irregularity appears with the next higher salinity showing an increase in egg survival.

If, now, the per cent mortality occurring between two successive observations is studied for each salinity and age (time of each observation) the same data may be presented in a different manner and as a result another feature becomes evident (Table II). The table (with the exception of the 34.28‰ salinity) indicates lower mortalities in the lower salinities with optimum survival in the 11.55‰ groups. In addition, a greatly increased mortality occurs at 61 hr. and again at 204 hr. This is particularly evident in the optimum salinity of 11.55‰ and in the 6.06‰ salinity. As observations were made only on alternate days it is difficult to isolate the exact time at which the mortalities increased abruptly. However, there appear to be two critical stages in development. A preliminary experiment carried out in 1950 in order to determine the best culture methods likewise indicated critical stages in development. The 1950 data summarized in Fig. 1 show two sharp points of inflection in the mortality curve, between one and three days of incubation and between the 8th and 10th day of development. As will be discussed later the first surge in mortality is evidently related to the closure of the blastopore and the second occurs prior to hatching. Battle (2) found salmon eggs very susceptible to adverse conditions during these stages.

Observations indicated that the rate of development is the same in all salinities between 6.06‰ and 34.28‰. Optic lobes were apparent at 46 hr. and the initial movement occurred at 118 hr. in all of the salinities. Hatching commenced in all of the solutions on the 10th day and was completed by the 15th day. Careful observations on the hatching process indicated that the "normal" "head first" mode of hatching (the mode producing the greatest survival) which occurred over 80% of the time, took place in all but the 6.06‰ solution.

The mechanism of hatching appears to start with a noticeable softening of the egg membrane. Recently-dead eggs or eggs which are not yet ready to hatch, have an egg membrane which is comparatively difficult to break, even with a dissecting needle. An egg about to hatch has an egg membrane which is easily broken, even by jarring. The dorsal part of the embryo's head is then seen to push against the egg membrane thus forming a bulge on one side of the egg. The bulge is then forced farther and farther out until it eventually breaks. The head of the emerging embryo is now free of the egg and by a series of violent movements the yolk sac is forced out. The process of hatching is completed when the larva frees its tail by merely swimming out of the ruptured egg membrane. The entire process, observed in several cases, took approximately one hour and fifteen minutes. Although a few embryos started to hatch tail first these were never observed to emerge completely from the egg. Swimming movement of the tail seemed only to force the head farther into the egg shell. This was particularly apparent in the case of the 6.06‰ salinity. In this salinity, six days after hatching had commenced, there were only a half dozen live larvae swimming about the four beakers. Numerous dead larvae were found with only their tails protruding from the ruptured shells. Table I shows the number of larvae remaining alive in each salinity at the end of the hatching period. The percentage of the original number of eggs producing live larvae was very low, perhaps misleadingly low, as large numbers of the eggs did hatch but the mortality during and immediately following hatching was extremely high. Table I indicates that the 11.55‰, 16‰, and 34.28‰ salinity solutions produce the largest per cent survival of larvae six days after hatching had commenced. These same salinities produced the highest survival of eggs.

Experiments with Changing Salinity

Eggs for this experiment were stripped onto glass slides and treated as in the preceding experiment. In this case, however, each slide was scored with the diamond so that the egg slide could be readily broken into three pieces. Sixteen marked slides were placed into each of the eight salinities, four slides to each beaker, and left for at least two days. From then on, at intervals of approximately 48 hours, eggs were transferred from one salinity to another and left in that secondary salinity for approximately 48 hours. The transfers from each salinity were placed in an additional series of beakers containing fresh water of the appropriate salinity. For example, in the case of the 22.30‰ salinity, seven of the 1/3-egg slide sections were taken from one of the beakers at 22.30‰ and transferred to each of the other seven salinities. As a control, an eighth egg slide section was also removed from the initial culture beaker to another beaker of the same salinity. In this manner 56 slide sections were moved every second day. Theoretically there should have been a transfer of 64 slide sections, but all the eggs placed in the pond water (0‰ salinity) died during the first day. After 48 hr. in the secondary salinities each slide section was examined and observations made on development and

mortality. As development is rapid in the case of herring, it was felt that if eggs survived for two days in the changed environment they could be considered as having become acclimatized to their new environment.

Except for a few slides which were once more transferred to other salinities, the egg slides from the original transfer were discarded after examination.

Egg Density and Survival

The density of eggs on slide sections used in the varying salinity experiments varied between 10 and 200 eggs per section. The possibility that egg density might have an effect on subsequent survival was tested for significance by application of the Chi-square test (4, pp. 83–85). In this analysis a number of egg density groups were chosen and for each group two values were considered: (*a*) the total number of living eggs in each density group at the end of the salinity change (48 hr.) and (*b*) the total number of eggs which died in each density group during the 48 hr. A Chi-square value of 497 was obtained

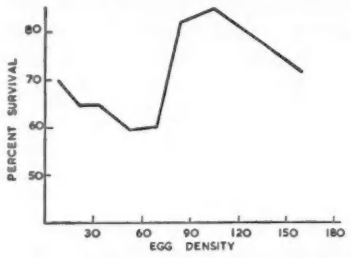

FIG. 8. Survival of herring eggs on slide sections at various egg densities.

from the analysis indicating that egg survival is not independent of density (tabled value at 0·01 *P* and 7 degrees of freedom being 18.48). Fig. 8 shows the average percentage survival for each egg density group plotted against the mid-point of each density group. There seems to be some evidence for the existence of an optimum survival around densities of 75 to 115 eggs per slide section. Quite obviously then, this feature might lead to large experimental errors, especially when it is recalled that all survivals are weighted by being converted to percentage survival. However, during the varying salinity experiment, slide sections of various egg densities were randomly distributed over the different salinities, i.e., no selection was made as to whether high or low density egg slides were placed in certain salinities or not. Also, average survivals are used in the analysis, therefore experimental error, resulting from differential egg survival caused by various densities, would seem to be held to a minimum.

Survival Following Transfer to Different Salinities

In Table III, the percentage survival of eggs is shown in relation to magnitude of salinity change. In this table percentage survival of eggs transferred, either up or down the range of salinities, from changes involving 0‰ (the

TABLE III

MAGNITUDE OF SALINITY CHANGE AND PERCENTAGE SURVIVAL AT 36, 90, 135, AND 190 HR.

	Salinity change in ‰													
	−34	−30	−25	−20	−15	−10	−5	0	+5	+10	+15	+20	+25	+30
At 36 hr.							0	68	66	85	67	26	22	96
						0	41	69	100	42	20	0	96	
					0	48	52	96	68	51	4	87		
				0	33	48	93	79	70	4	70			
			0	0	7	42	5	12	2	90				
		0	14	62	100	23	61	9	80					
	0	86	75	90	22	37	22	97						
At 90 hr.							61	84	81	83	96	84	60	92
						87	86	100	100	87	94	97	86	
					59	100	95	89	79	72	53	51		
				46	86	98	98	74	77	66	75			
			89	75	92	92	100	97	86	94				
		18	97	100	86	97	100	21	92					
	95	98	99	100	89	95	84	86						
At 135 hr.							31	77	78	96	96	73	8	87
						69	100	95	100	100	96	42	70	
					87	96	100	94	98	64	25	—		
				36	98	90	93	89	100	36	100			
			5	36	100	100	71	100	85	84				
		7	100	100	100	83	100	0	70					
	50	51	100	100	89	95	84	98						
At 190 hr.							67	84	39	63	81	18	67	49
						38	98	64	81	50	71	53	78	
					19	59	96	100	57	79	71	82		
				0	76	29	74	30	19	72	78			
			0	50	43	64	85	34	58	66				
		4	60	81	62	96	80	—	—					
	65	93	92	91	97	88	74	81						
Average	70	45	61	60	68	69	70	71	73	69	59	51	61	81

control) to + or − 30‰ or 34‰ salinity are shown. It can be seen that the survival of eggs on the control sections was not necessarily the highest. In the 27 control slide sections, survival on these sections as compared to the other changes, was higher in 7 cases, lower in 8 cases, and about average in the remaining 12 cases. On this basis alone, it appears that changing from one salinity to another produces no higher mortality than a change involving no salinity difference. Further, it is apparent from the average value for the four experiments that there is no obvious correlation between survival and magnitude of salinity change within the range of salinities under consideration. With but two exceptions the results show that the per cent survival of eggs in salinity changes ranging from +30‰ to −34‰ varies only between survivals of 60% and 73%. The two exceptions indicate survivals of 51% and 45%.

If magnitude of salinity change, then, has no apparent relation to survival, the average survival in the various secondary salinities, regardless of

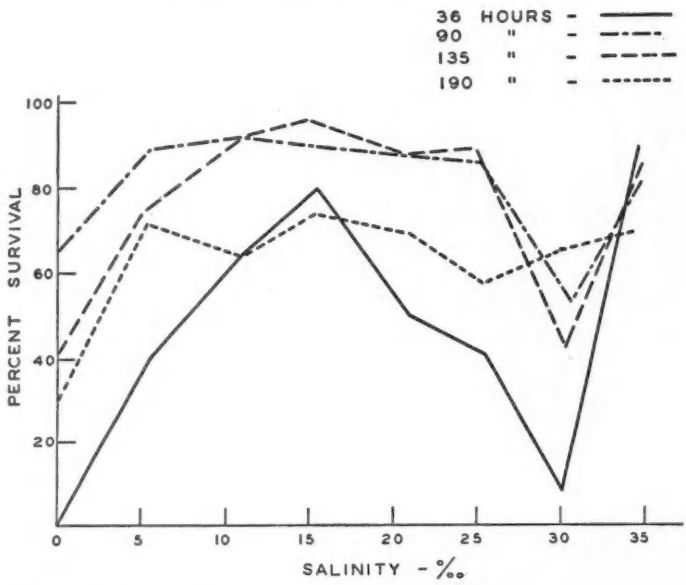

FIG. 9. The survival of herring eggs transferred at the indicated ages into various salinities for a period of 48 hr.

the change which placed the eggs there, can be considered. In Fig. 9 the average percentage survival is shown for eggs taken at various ages from each of the acclimation salinities and placed for approximately 48 hr. in the indicated secondary salinities. A number of features are indicated. In the first place, survivals at all ages are lowest in the 0‰ and 30.26‰ solutions. Secondly, there is an abrupt increase in survival in all cases in the 34.28‰ salinity. Also, it appears that during the initial changes at 36 hr., there is a considerable narrowing of the tolerance range, with survivals grouped about an optimum of 16.24‰. During the changes at 36 hr. there was no survival of eggs placed in pond water (0‰). Fig. 9 also indicates that during later development, the embryo is apparently capable of tolerating a wider range of salinities—even to the extent of tolerating, at least for 48 hr., fresh water. Finally, it seems significant that there is a lower survival during the initial (36–86 hr.) change and in the final (190–237 hr.) change. These are periods of development which would correspond to blastopore closure, and the pre-hatching periods. Perhaps this is another illustration of increased mortality during the critical periods previously discussed.

Table IV represents the survival of eggs taken from the secondary salinities and transferred once again to either higher or lower salinities. The percentage survival in each change indicates once again a high tolerance. The controls show no consistently higher survival and the magnitude of the change indicates no apparent relationship with survival.

TABLE IV

PERCENTAGE SURVIVAL OF EGGS THROUGH TWO SALINITY CHANGES; AGES 37, 87, 132 HR.

Salinity, ‰			Percentage survival	
Original	First transfer	Second transfer	First transfer	Second transfer
6.06	6.06	6.06	67	45
16.24	11.55	6.06	52	50
34.28	22.30	6.06	89	79
30.26	16.24	6.06	100	68
22.30	16.24	6.06	89	75
22.30	22.30	22.30	79	25
11.55	16.24	22.30	100	84
6.06	11.55	22.30	66	38
34.28	26.13	22.30	37	29
34.28	34.28	34.28	97	86
16.24	22.30	34.28	68	39
6.06	16.24	34.28	85	10

Development in the Secondary Salinities

Development in salinities between 11.55‰ and 34.28‰ seemed to be identical. In 6.06‰ activity prior to hatching was less than in the higher salinities, and in the 0‰ salinity, although the embryos survived the salinity change, there was little if any movement after 90 hr. During the stage of eye pigmentation, the embryos in this solution had little, if any, pigmentation, whereas at the same age in higher salinities the eyes were heavily pigmented.

Salinity Tolerance of Larvae

Using the procedures outlined previously no difficulties were encountered in rearing the majority of the herring larvae past yolk sac absorption. By the end of the sixth day after hatching most of the larvae had completely absorbed their yolk sacs and many could be seen actively pursuing brine shrimp nauplii. At this time many of the herring larvae were noted to have numbers of nauplii within their intestines. No abnormalities were noted and growth and activity seemed to be the same in all of the salinities between 6.06‰ and 34.28‰.

Twenty larvae (13–16 days after hatching) were taken from the 34.28‰ salinity and placed into a rearing jar of pond water (0‰ salinity). Three hours later these larvae were all dead. From the limited observations it would appear that herring larvae have a salinity tolerance range which extends, at least, between 6.06‰ and 34.28‰. The determination of an optimum salinity range is obviously impossible with such small numbers. Further work along this line seems warranted as the mechanism of osmoregulation in the herring larvae would seem to be well developed for life in both brackish and salt water.

Discussion

For many teleosts osmoregulation is exercised only within narrow limits. Extent of salinity tolerance varies considerably among different species. As the Pacific herring spawns inshore where salinities vary considerably (8.21‰ to 27.68‰ on some spawning grounds such as Queen Cove, Vancouver Island, B.C.) it would seem that this particular fish must have a high salinity tolerance. Nash (8) showed in the case of two marine clupeoid fishes, *Sardinella macrophythalmus* and *Sardinella anchovia*, that the kidneys had a glomerular count approaching that of fresh water teleosts. Kyle (6) states that the herring is one of the most primitive of fishes and has only been a marine form since the Cretaceous period. It seems probable that the Pacific herring has a relatively unspecialized teleost kidney and, at least in part, tolerates brackish water because of its high glomerular development.

The mechanism of osmoregulation in fish eggs has been less intensively studied than in fish. However, a few features have been demonstrated. Vernidub (12) found that the reaction of developing fish eggs to external factors varies at different stages of development. At some stages there is no evident effect on morphogenesis (division, gastrulation, growth of the developing embryo, or pigmentation of the eyes) while at other stages, the same factor may produce marked effects resulting in death of eggs. Battle (1) showed that changes in salinity will so alter the physiological processes concerned with growth and differentiation of the rockling that the embryo develops abnormally. Ford (5) found that the European herring showed a high salinity tolerance for normal development and hatching. Apparently then different developmental stages as well as different species of fish react differently to changes in the environment.

Results of the salinity experiments have shown the existence of a wide salinity tolerance for eggs and larvae of the Pacific herring. In conditions of different constant salinities, the range is from 6.06‰ to 34.28‰. There seems to be a trend toward higher survivals in salinities of 11.55‰ and 16.24‰. An explanation is offered below for the unexpectedly high survival of eggs held at 34.28‰. Experiments in which eggs were moved from one salinity to another at various ages also indicated a wide salinity tolerance with a marked increase after the first two days of development. Developing eggs of later ages will survive in pond water of 0‰ salinity for at least two days.

Ford's (5) observations on *Clupea harengus* are in agreement with the results obtained for *Clupea pallasii*. He showed that a high egg survival was obtained over a wide range of salinities, 4.8‰ to 37.5‰. In the low salinities, 4.8‰ and 9.3‰, many larvae were unable to wriggle free of the egg envelope. In the present experiment this appeared to be the case in the 6.06‰ salinity, but hatching was normal in all other salinities. This inability to hatch beyond a mere protrusion of the tail from the egg capsule was noted by Battle (1) in her studies of the four-bearded rockling. She related this to an inadequate development of the tail musculature.

Ford indicated successful fertilization of European herring eggs in salinities as low as 4.8‰. In the present experiments, eggs were successfully fertilized in all salinities from 6.06‰ to 34.28‰. These observations suggest that only a very low salt concentration is necessary to produce successful fertilization and subsequent development.

Since it is apparent that the eggs are capable of tolerating a wide range of salinity, one might deduce that the egg membrane is impermeable to water and thus no problems of osmoregulation exist. This hypothesis does not, however, explain all of the facts. Developing eggs at early stages die within a few hours when placed in pond water, but at a later age eggs will survive for several days when placed in the same medium. As Rollefsen (10) has shown for cod eggs, it may be the growth of the embryonic area (impermeable to water in cod eggs) over the yolk which explains how herring eggs are able to tolerate fresh water at a later stage of development. If this embryonic layer, in the case of herring eggs, is water impermeable, then it might be expected that as the egg developed the tolerance range would increase. The results indicate that following blastopore closure there is a considerable widening of the tolerance range.

The more or less well defined optimum salinity (11.55‰–16.24‰) for development and hatching is of interest. This might perhaps be expected if the herring egg is permeable to water. Sea water of normal salinity, around 35‰, has an osmotic pressure which is two and three times as great as that of marine teleosts (3). Thus, the salinity which would be isotonic as far as the herring egg is concerned would probably be within the range of 11.55‰ to 16.24‰ salinity. In sea water within this range then, eggs would have little or no osmoregulatory problems and therefore would probably be in a better position to survive. This may also provide an explanation as to why herring spawn in a locality such as Queen's Cove where salinities have been shown to vary from 8.2‰ to 27.68‰.

In the 1950 preliminary experiments it was noted that egg slides during the course of the experiment became covered with a brown, filamentous, granular layer. Unfortunately it could not be identified beyond the observation that it appeared to be some form of fungus growth. In 1951 the same growth was apparent in all but the 34.28‰ solution. In this salinity the egg slides remained clear throughout the experiment. On the basis of these observations, it is suggested that the fungus growth produces a considerable mortality in salinities between 6.06‰ and 30.26‰. In the case of the 34.28‰ solution, however, the salt inhibits growth of the fungus.

In spite of this inconsistency, it is evident that Pacific herring eggs have a wide range of salinity tolerance and that this range increases with age. Optimum egg survival evidently occurs in salinity ranges (10‰ to 15‰) which are near isotonic for marine teleosts. The closure of the blastopore and the period just prior to hatching are critical stages in development and hatching is often abnormal in salinities lower than 6‰.

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THE MOSQUITOES OF SASKATCHEWAN[1]

By J. G. Rempel[2]

Abstract

This publication deals with the adult mosquitoes of Saskatchewan. Keys to genera and species are given. Six genera are represented in the mosquito fauna, namely: *Aedes* with 25 species; *Culex* with three species; *Culiseta* with five species; *Anopheles*, *Mansonia*, *Psorophora*, and *Wyeomyia* each with one species. Each species is described and important taxonomic characters are illustrated. Brief notes on biology and distribution follow the description of each species. The topography, climate, and vegetation of the province is discussed in relation to the distribution of mosquitoes. The seasonal distribution of the mosquitoes in the three main ecological zones, namely prairie, aspen grove zone, and coniferous forest zone, is outlined. Brief sections are devoted to mosquito adaptation to survival and to mosquitoes in relation to Western equine encephalomyelitis.

Introduction

Since the end of the second world war there has been an increasing interest in the biting flies of our north country. This has led to the fine work of Twinn, Brown, Jenkins, and Hocking, to mention but a few, in northern Manitoba, in the Yukon, and in Labrador. The most important biting flies are, of course, the mosquitoes and they have received the major share of attention. These studies by Canadian and American workers have added much to our knowledge of arctic mosquitoes. The species studied have been largely those found in the Northern Transition Forest zone and on the tundra. Since the investigations in Saskatchewan deal with the species ranging from the coniferous forest zone down into the prairies, they supplement the above studies. Moreover, Saskatchewan may be looked upon as a transition zone from north to south and since the mosquito fauna of the United States has received intensive study, a knowledge of our species would seem to fill an important gap. The study is limited to the prairie forms and to the species of the coniferous forest zone in the central part of the province. The northern half of the province, the area within the Canadian Shield, still awaits investigation. This publication deals only with adult mosquitoes since the larvae of Saskatchewan species were treated in the writer's *Guide to Mosquito Larvae of Western Canada* (34).

History

The first entomologist to study the Saskatchewan mosquito fauna was T. N. Willing, an ardent collector of insects and plants. There is, however, no published record of his observations. In 1907 Frederick Knab spent several months at the town of Oxbow studying prairie mosquitoes. He made observations on seven species and published a fairly extended account on the bionomics of some of the species, especially *Aedes spencerii* (20). In 1918

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A. E. Cameron studied the mosquitoes in the Saskatoon area and published a short account of his findings (2). During the summers of 1926 and 1927 E. Hearle (13), while stationed at Indian Head, made a detailed study of the biology of *A. flavescens*. In the spring of 1929 Dr. K. M. King and his staff of the Dominion Entomology Laboratory at Saskatoon undertook a survey of the mosquito situation in the newly opened Prince Albert National Park with a view of determining methods of control. During that summer and in the following year Dr. King made extensive collections of larvae and adults in the park and submitted the material to Mr. E. Hearle for identification. This study added materially to our knowledge of our woodland species. In the summer of 1938 the writer made further collections of mosquitoes at Waskesiu and carried out extensive rearing.

Following the epidemic of encephalomyelitis in the summer of 1941, it became evident that a study of the prairie species was urgently needed. From 1942 to 1952 the writer made such a study and some of the findings are reported here. Other data have been published elsewhere (34, 35).

Since the 1941 outbreak of encephalomyelitis was heaviest in the triangle between Regina, Moose Jaw, and Weyburn (Fig. 159), it was decided to concentrate work in the Regina district. Although the investigation was intensive rather than extensive, other areas, including many localities in the aspen grove region, were visited from time to time.

During the summer of 1952 a number of university students engaged in biological or geological field studies in various parts of the province made extensive larval collections for the writer. Through this co-operation, knowledge of the geographic and seasonal distribution of our species was markedly extended.

Classification

The identification of northern species of mosquitoes is a difficult task. The specimens most easily encountered are the "rubbed" females, which are, however, the most difficult to identify. The males and the larvae of mosquitoes have excellent diagnostic characters. But males are short-lived and are not attracted to man. To secure good specimens one must resort to rearing. The larval stage is passed through quickly and is therefore frequently missed. Hence the student must often work with specimens which, taxonomically, present the greatest problem. To ease his task the descriptions given here are accompanied by extensive illustrations based on unrubbed specimens. Whenever possible the drawings have been made from reared material.

It cannot be emphasized too strongly that anyone engaged in a study of mosquitoes must make every effort to secure larvae and rear the adults. The last larval skin, the male genitalia, and the unrubbed female greatly simplify the task of identification.

The terminology used by the writer is based largely on that used by Ross in his *Mosquitoes of Illinois*. The reader unfamiliar with the terms should consult the illustrations in Figs. 1 and 2.

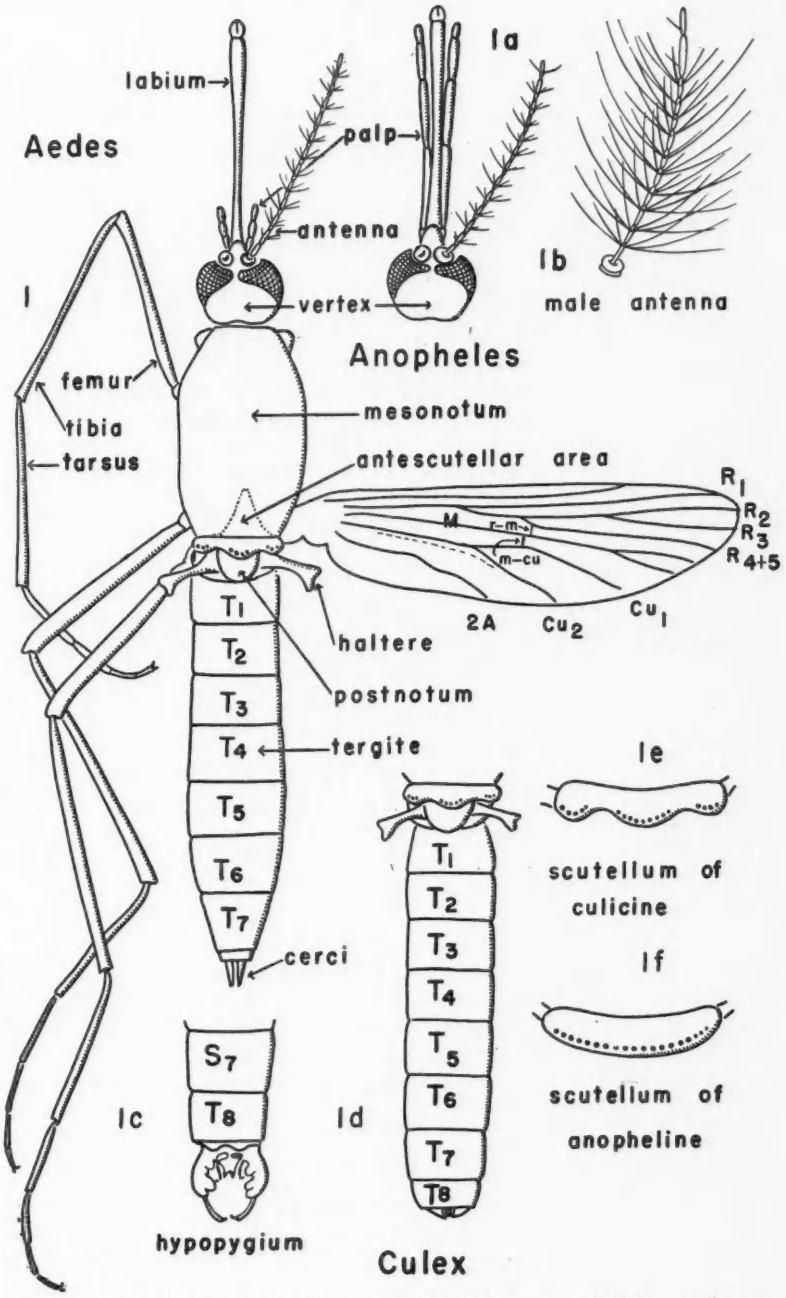


FIG. 1. Diagrams of anatomical features used in the taxonomy of adult mosquitoes.

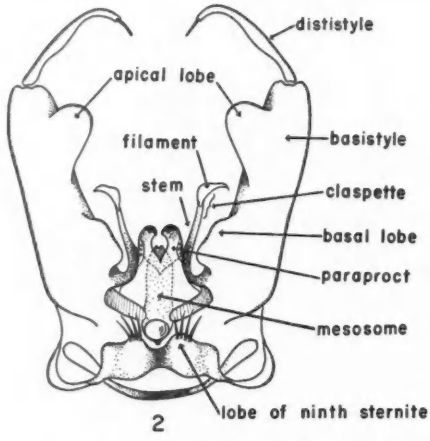


FIG. 2. Hypopygium of *Aedes canadensis* to show nomenclature of parts.

KEY TO GENERA

Females

1. Scutellum evenly rounded, crescent-shaped, Fig. 1*f*.....*Anopheles*
   Scutellum trilobed, Fig. 1*e*..... 2
2. Postnotum with a tuft of setae.....*Wyeomyia*
   Postnotum without a tuft of setae..... 3
3. Abdomen pointed; cerci long and narrow, Fig. 1.....*Aedes*
   Abdomen blunt; cerci not long and pointed, Fig. 1*d*..... 4
4. Fork of R_{2+3} and fork of M equidistant from base of wing, Fig. 29..... 6
   Fork of R_{2+3} and fork of M not equidistant from base of wing..... 5
5. Fork of R_{2+3} closer to base of wing than fork of M, Fig. 24.....*Culex*
   Fork of R_{2+3} more distant from base of wing than fork of M.....*Psorophora*
6. Wing not spotted, scales broad, Fig. 4; cross veins *r-m* and *m-cu* far apart; first tarsal segment with broad white ring near the middle.....*Mansonia*
   Wing frequently spotted, scales narrow; cross veins *r-m* and *m-cu* in line or only slightly separated.....*Culiseta*

KEY TO GENERA

Males, based on terminalia

1. Dististyle contorted, with several lobes and many spines, Figs. 6, 7.....*Wyeomyia*
   Dististyle not contorted, generally unlobed, ending in a single claw..... 2
2. Claspette present, generally ending in a sclerotized filament, Fig. 2. Most species of..*Aedes*
   Claspette absent, or if present not ending in a sclerotized filament..... 3
3. Dististyle inserted before apex of basistyle, Fig. 138.....*Aedes*
   Dististyle inserted at apex of basistyle..... 4
4. Basistyle with two prominent parabasal spines situated on short tubercles, Fig. 8..*Anopheles*
   Basistyle not as above..... 5
5. Basal lobe with a rodlike spine, Fig. 5.....*Mansonia*
   Basal lobe absent, or if present, not bearing a rodlike spine..... 6
6. Basistyle with a subapical lobe bearing rods, spines, and/or a leaflike blade, Figs. 10, 15*b*, 18.....*Culex*
   Basistyle not as above..... 7
7. Dististyle gradually tapering toward apex, Figs. 42-45.....*Culiseta*
   Dististyle widening at the middle, Fig. 3.....*Psorophora*

Genus **Psorophora** Robineau-Desvoidy

This genus is represented in Saskatchewan by one species.

Psorophora signipennis (Coquillett)

Fig. 3

Description

Proboscis black with a broad diffuse median white ring. Mesonotum dark brown with silvery scales, without distinct pattern. Abdomen with black and white scales, the black scales forming a V, with apex directed forward. Wings with black and white scales; costa white with two black patches; fringe alternately black and white. Front leg: femur with black and white scales and preapical white ring; tibia with black and white scales intermingled; first tarsal segment mainly white with black apical band, second segment with narrow white basal band, remainder all black. Hind leg: femur as in front leg; tibia with black and white scales intermingled, black ring at base and at apex; first tarsal segment mainly white with near basal and apical black ring; second, third, and fourth tarsal segments with basal half white; last segment with narrow basal white ring.

Hypopygium (Fig. 3). Dististyle slightly distended near the middle, with reticulation toward apex, outer and inner margin with a few setae. Basistyle with long hairs on outer margin and with short, curved setae on the upper surface; apical lobe poorly developed; basal lobe with a half a dozen setae.

Notes on Biology and Distribution

Little is known of the biology of this species. According to Dyar (3) it is adapted to arid conditions of the plains and larvae are found in small transient rain pools. Rozeboom (37) also records larvae from artificial containers. According to Mail (24) development is very rapid, the period from egg to adult being completed in five days.

This species occurs from the plains of southern Canada to Mexico. The writer has in his collection one male specimen collected at Regina, July 8, 1942. In view of the extensive collecting in the Regina area over a period of six years, this scarcity of material indicates that the species is extremely rare. According to Mail (24) it is comparatively rare in Montana. Rozeboom (37), however, finds it to be one of the commonest species in Oklahoma.

Observations regarding its feeding habits are conflicting. Mail found that the adults bite readily, while Rozeboom states that in spite of their abundance, adults were rarely observed biting.

Genus **Mansonia** Blanchard

The synonymy of this genus is still in dispute. In general, American workers have for some time used the name *Mansonia*, while European workers use the name *Taeniorhynchus*. In this respect Marshall (25, p. 107) comments as follows:

> "The relative merits of the names *Taeniorhynchus* and *Mansonia* (which apply to the same genus of mosquitoes) have been much disputed, but the members of the Committee on Generic Nomenclature of the Royal Entomological Society of London recently gave their unanimous opinion that it is more correct to use the former name."

The writer, however, follows the custom of the leading American workers, in adopting the name *Mansonia*.

The immature stages of the species of this genus differ markedly from those of other species in their adaptation to secure oxygen from plants. The siphon of the larva and the respiratory trumpet of the pupa are modified for piercing plant tissues.

In Saskatchewan this genus is represented by one species.

Mansonia perturbans (Walker)

Figs. 4 and 5

This species is rather easily separated from all other Saskatchewan mosquitoes on the basis of the following characters: the first tarsal segment has a broad median white ring; the wing scales are broad and cover the major part of the anterior half of the wing surface.

Description

Head with golden bristles; proboscis with black and yellow scales, the latter predominating near the base and near the middle. Mesonotum brown with golden hairs and two longitudinal bare stripes. Front leg with black and white scales intermingled on femur and tibia, with narrow white rings at tip of femur and tibia, and broad ring at basis of each tarsal segment and at middle of basal tarsal segment; middle leg similar to front leg; hind leg with additional broad white ring near middle of tibia. Wing scales (Fig. 4) broad, white and black intermingled, covering the major part of the wing surface. Abdomen dorsally covered with dark scales, laterally white scales form small triangular patches near the anterior margin, venter with white and black scales, the former predominating on the anterior half of each segment.

The hypopygium (Fig. 5) is easily distinguished from that of any other of our species. The dististyle is distally enlarged and has a twisted appearance; the apical lobe is absent; the basal lobe has a fine hairlike spine and a blunt rodlike spine; the ninth tergite has two broadly separated lobes each bearing five to eight spines.

Notes on Biology and Distribution

Although a common and widespread mosquito in North America, *Mansonia perturbans* is rarely encountered in Saskatchewan. The writer has collected a few adults in the vicinity of a small pond in the Prince Albert National Park, (July, 1948). Another record for the province is: Waskesiu Narrows, July 21 1930 – K. M. King.

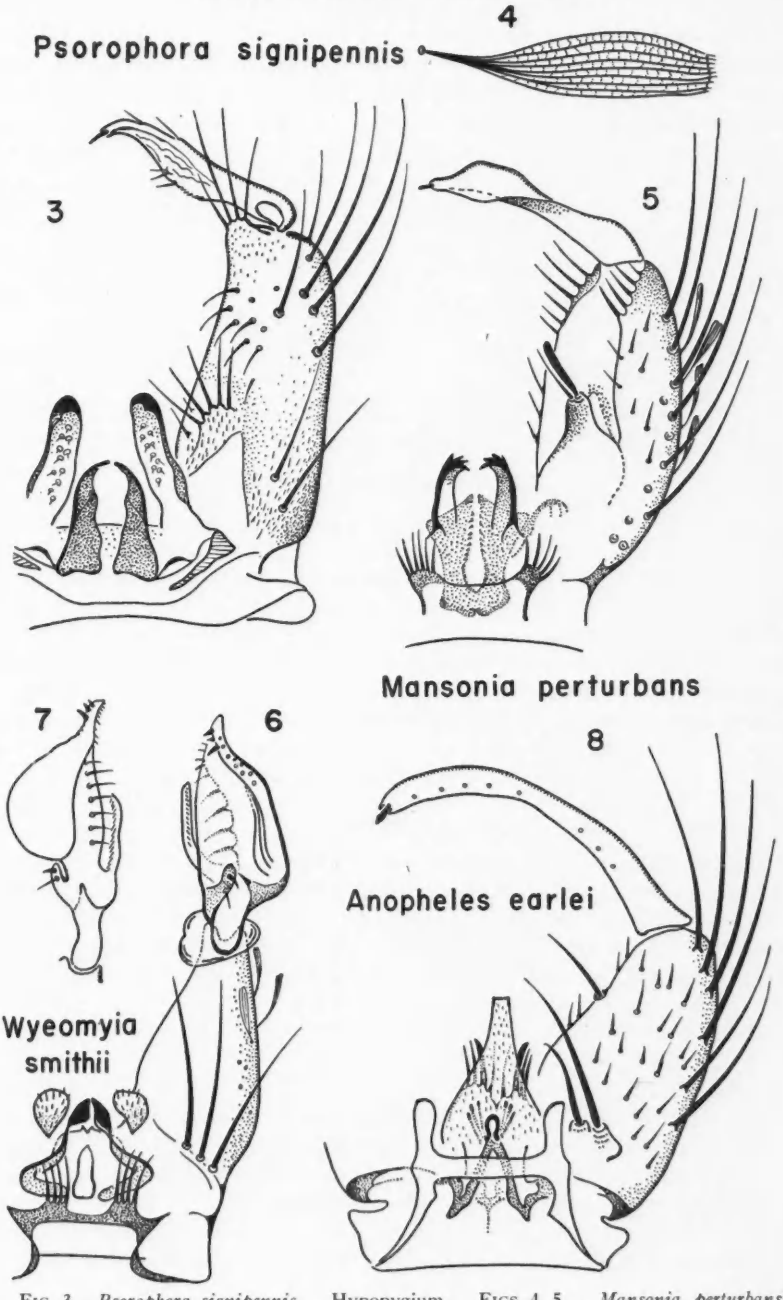


FIG. 3. *Psorophora signipennis*. Hypopygium. FIGS. 4, 5. *Mansonia perturbans*. 4, scale of wing; 5, hypopygium. FIGS. 6, 7. *Wyeomyia smithii*. 6, hypopygium; 7, lateral aspect of dististyle. FIG. 8. *Anopheles earlei*. Hypopygium.

The immature stages of this species are not readily collected because of their habit of remaining permanently under water. The larvae and pupae are known to attach themselves to stems or roots of aquatic plants and remain attached for the greater part of the year. The winter is passed in the larval stage. There is one generation a year, the adults appearing in late spring and summer.

It is generally agreed that this species is a fierce biter. Hearle (12) describes the species as follows:

> "It is an extremely vicious biter and the attention of a few *M. perturbans* Walk., can create more discomfort than the bites of large numbers of most other species. No other mosquito that we are acquainted with has such virulent poison. It shows no timidity in attack and is very persistent in entering houses."

Because of its limited distribution and scarcity, it is of little importance in Saskatchewan.

Genus **Wyeomyia** Theobald

The members of this genus are mainly tropical and subtropical mosquitoes which breed in the water of bromeliads. Only three species are found in North America and one of them, *Wyeomyia smithii* (Coq.), extends into Canada.

The most easily recognized distinguishing characteristics of this genus are the small size, the presence of bristles on the postnotum, the presence of broad appressed scales on the mesonotum, short palpi in the male, and conspicuous silvery scales on the venter of the abdomen.

Wyeomyia smithii (Coquillett)

Figs. 6, 7

This is the so-called pitcher plant mosquito. The larvae are found exclusively in the pitcher plant *Sarracenia purpurea* L. This species has not yet been recorded from Saskatchewan. However, since its host plant is found in the province, and since the species is found in Manitoba, it was deemed advisable to include it here. The writer has on loan three specimens from the Eric Hearle collection at Kamloops, B.C., that bear the label: Aweme, Man., 7-VII-25, R. M. White.

Description

A very small mosquito. Proboscis dark-scaled. Vertex with brown scales, genae with silvery scales. Mesonotum with dark brown appressed scales; postnotum with a tuft of bristles; pleurae with conspicuous silvery scales. Abdomen dorsally dark brown, ventrally silvery white. Wing scales all dark. Outer surface of legs dark scaled, inner surface white scaled. Distal half of second and all of third and fourth tarsal joints of mid-tarsus white scaled.

Hypopygium (Figs. 6, 7). Dististyle enlarged and distorted beyond middle, its lobes provided with spines or setae. This character immediately separates the species from all of our other species.

Notes on Biology and Distribution

This species has a wide distribution in North America. It has been recorded from various eastern states, from Labrador and Ontario, and from as far west as Minnesota. Its geographic distribution probably coincides with the geographic distribution of the pitcher plant.

The larvae are found only in the leaves of the host plant. There are several generations a season. The species overwinters in the larval stage.

At Goose Bay, Labrador, Haufe (11) found larvae present throughout the summer. "Even when temperatures were near the freezing point and ground pools were still covered with snow and ice, the larvae were observed to be active in the taller, pitcher-shaped leaves that were exposed sufficiently to the sun during the day to have their contents thawed completely or in part", p. 256.

The adult of *Wyeomyia smithii* is not known to take blood.

Genus **Anopheles** Meigen

Only one species of this genus occurs in Saskatchewan, namely *Anopheles earlei* Vargas. Our earliest references to this anopheline mosquito are given under *A. maculipennis* Mg., while later ones under *A. occidentalis* D. and K. Vargas (43) described the eastern American form of the *maculipennis* group as *A. earlei*. After a careful study of all stages, Vargas and Matheson (44) have come to the conclusion that these North American forms should be considered as two species: *Anopheles earlei* Vargas with a distribution in southern Canada and northern United States extending from the Atlantic west to the Rocky Mountains, and *A. occidentalis* D. and K. west of the mountain range. Pratt (31) discusses the American species of the *maculipennis* complex and indicates their distribution on a map. It is clear that *A. earlei* extends much further north than indicated here, for the species has been reported from Labrador by Haufe (11) and from the Yukon and Alaska by Jenkins (personal communication). Dr. Matheson kindly examined a series of adult anophelines from Saskatchewan and identified them as *A. earlei*.

Anopheles earlei Vargas

Fig. 8

Description

The generic characters of the anopheline mosquitoes given in the key will readily separate the species from all other species of the province. In addition, the arrangement of the wing scales to form spots over the cross veins and at the forks, plus the coppery spot on the fringe at the apex, constitute easily seen diagnostic characters.

Hypopygium (Fig. 6). The male genitalia are of the general anopheline type.

Notes on Biology and Distribution

This species overwinters as the adult female in a place where it receives shelter from the rigors of the cold weather. It is frequently observed in homes during the winter or early spring and may then form the subject of startling news reports in the local press. The larvae are generally found at the edge of small slow-flowing streams where there is considerable aquatic vegetation. The adults are fierce biters but because of small numbers they are of little importance in this province.

In Saskatchewan this species has a wide distribution, having been encountered on the open prairie in the Regina district, in the aspen grove region from Saskatoon to Prince Albert, and in the northern wooded areas of Waskesiu and Lac la Ronge. In general the species is quite rare in the open southern areas, but can be encountered in fair numbers in localized wooded areas of the north. Adult females are found on the wing in late May or early June, while larvae may be found in July. The writer has the following locality records for the province: Regina, Qu'Appelle, Stockholm, Maple Creek, Saskatoon, Redberry, Turtle Lake, Prince Albert National Park, and Lac la Ronge.

Genus **Culex** Linnaeus

The members of the genus *Culex* are found mainly in tropical and subtropical regions where, according to Matheson (26), more than 300 species occur. For North America north of Mexico some two dozen species have been described. Of these only three species have been collected in Saskatchewan. The latter are readily separated.

Key to Females

1. Tarsi ringed with white ..... *tarsalis*
   Tarsi not ringed with white 2
2. White abdominal bands on anterior margin of segments ..... *restuans*
   White abdominal bands on posterior margin of segments ..... *territans*

Key to Males, Based on Terminalia

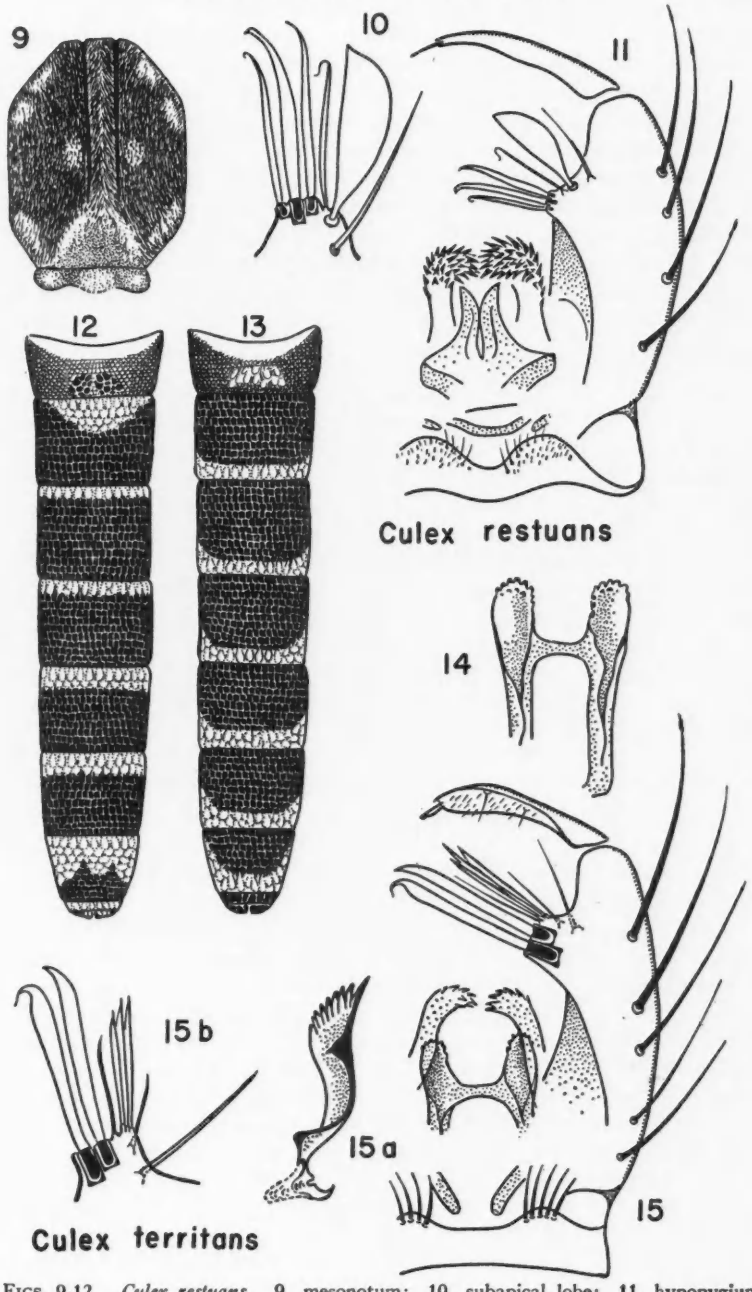
1. Paraprocts with a single marginal row of teeth, Figs. 15 and 15*a* ..... *territans*
   Paraprocts with numerous rows of teeth 2
2. Subapical lobe with two stout rods, three setae, and a narrow leaf, Figs. 17 and 18 ..... *tarsalis*
   Subapical lobe with four flat apically curved spines, one seta, and one broad leaf, Fig. 10 ..... *restuans*

Culex restuans Theobald

Figs. 9, 10, 11, 12

Description

Proboscis dark brown, with scattered white scales on under side. Vertex with fine white appressed hairs and numerous erect flat brown scales. Mesonotum (Fig. 9) dark brown, covered with bronzy brown hairs which are interrupted by two longitudinal bare lines; white patches as follows: near anterior lateral margin, above wing bases, on either side of the antescutellar area and near center of disk. Scutellum with white hairs. Abdominal segments (Fig. 12) ringed basally. The white scales on segment two in the form



FIGS. 9-12. *Culex restuans*. 9, mesonotum; 10, subapical lobe; 11, hypopygium; 12, abdomen. FIGS. 13-15. *Culex territans*. 13, abdomen; 14, mesosome; 15, hypopygium; 15*a*, paraproct; 15*b*, subapical lobe.

of a triangular patch, segment seven with white band broad and extending posteriorly along the mid line and at the sides, segment eight entirely white scaled. Legs largely dark scaled along outer surface, with numerous white scales along inner surface, tips of femora and tibiae white.

Hypopygium (Figs. 10, 11). The subapical lobe bears six appendages, as follows: a group of three long flat spines, arising close together on the lobe, a smaller apically curved spine placed distally to this group, a leaflike appendage, followed by a slightly curved, sharp-pointed seta. Mesosome heavily sclerotized. Paraprocts distally provided with numerous prominent spines. Lobes of ninth tergite broad, covered with fine hairs and with small setae along inner margin.

Notes on Biology and Distribution

This species is common east of the Rockies and has been recorded from southwestern California. McLintock (22) considers this species (identified by him as *C. territans* Walker) numerically to be the third most important species in Manitoba, making up approximately twenty per cent of the mosquito fauna. In Saskatchewan the species appears to be rare. Our only records are two female specimens collected by the writer at Regina, Saskatchewan, and at Craven, Sask. No doubt the species is present in other parts of the province, especially the eastern section. It would appear from our records, however, that this province forms the limit of its westerly distribution. Strickland (40) does not report the species from Alberta.

The larvae of *C. restuans* are said to prefer small dirty pools and artificial containers. In Manitoba water barrels appear to be the favorite breeding place. The adult hibernates.

Culex territans Walker

Figs. 13, 14, 15, 15*a*, 15*b*

The synonymy of this species has been the subject of much disagreement. To quote Bohart (1, p. 334):

> "This species was called *territans* Walker in the earlier literature because the original description referred to apical abdominal bands and gave "United States" as the type locality. Theobald redescribed the female type, stating that the apical bands were "very indistinct in the specimen". He also cited "United States" as the locality. Edwards (1932) raised doubt as to the identity of *territans*. . . . . Considering Walker's and Theobald's definite statements as to the abdominal banding and type locality, with neither of which the supposed type agrees, it seems best to apply *territans* to the most common and widespread species of the *apicalis* group and to assume that the type has been lost."

Description

Proboscis dark brown, with few white scales on lower surface. Vertex with fine white hairs. Mesonotum brown clothed with fine golden hairs interrupted

by two narrow longitudinal lines; faint indication of two white spots near center of disk. Abdomen (Fig. 13) with apical white bands, narrow near the middle and widening near the sides. Wing scales dark brown. Legs with numerous white scales on coxae and basal two-thirds of femora; tip of femora, tibiae, and base of hind metatarsus white, remainder dark scaled.

Hypopygium (Figs. 14, 15, 15*a*, 15*b*). Dististyle expanded toward apex, with numerous fine hairs and two prominent hairs; claw expanded at tip. Subapical lobe (Fig. 15*b*) with two long and prominent blades, about equal in length, and six setae, three of which are somewhat bladelike and have their bases close together. Lobes of mesosome (Fig. 14) with blunt teeth, bridge sclerotized. Paraprocts (Fig. 15*a*) with an apical row of about nine teeth. Lobes of ninth tergite small and far apart, each with four or five marginal setae.

Notes on Biology and Distribution

This species (Bohart (1)) has a wide distribution in the United States. It is also found in Alaska. Twinn (41) records the species (as *Culex testaceus* van der Wulp) from Quebec. McLintock (22) found it (as *Culex apicalis* Adams) in the Winnipeg area. According to Hearle (12) the species (*C. testaceus* v.d.W.) is fairly common in localized areas of the Lower Fraser Valley of British Columbia. In Saskatchewan the writer collected larvae in a shallow, weedy, permanent pool at Lac la Ronge, on June 5, 1948. He has seen one adult collected by Dr. L. G. Saunders at Saskatoon on July 7, 1930. No doubt the species has a much wider distribution in the province, but because of its restricted breeding and feeding habits, it is not generally collected. The female hibernates. In Alaska, Jenkins (15) found overwintering females throughout the month of May.

The larvae prefer clear water in grassy permanent ponds. It is generally believed that the adults feed on amphibians and do not attack man.

Culex tarsalis Coquillett

Figs. 16-24

Description

This is a strikingly marked mosquito. Proboscis brown with a broad white band near the middle. Antennae brown with prominent white scales on pedicel. Palpi brown with white scales at apices of last two segments. Vertex with white hairs along margin of eyes and along median line; nape with numerous erect white and brown scales. Mesonotum (Fig. 16) with dark brown integument covered by narrow bronzy brown scales, interrupted by two narrow bare lines; white scales along anterior and lateral margins, in the form of two white stripes or spots on disk, and on sides of the antescutellar area. Abdomen of the male (Fig. 19) with broad basal white bands, the one on second segment prolonged medially into a bar; abdomen of female (Fig. 20) similar to that of male; venter largely white scaled with black V-shaped markings (Fig. 21). Wing scales black with a few white scales on base of

costa; stem of R_{2+3} short, *m-cu* not in line with *r-m* (Fig. 24). Legs black scaled with prominent apical white rings on femora and tibiae, with basal and apical rings on most tarsal segments, and with a row of white scales on sides of femora, tibiae, and first segment of hind tarsus (Fig. 23).

Hypopygium (Figs. 17, 18, 22). Subapical lobe with two rods, three setae, and a blade; paraprocts (Fig. 22) with numerous spines; mesosome (Fig. 17) divided into bladelike processes.

Notes on Biology and Distribution

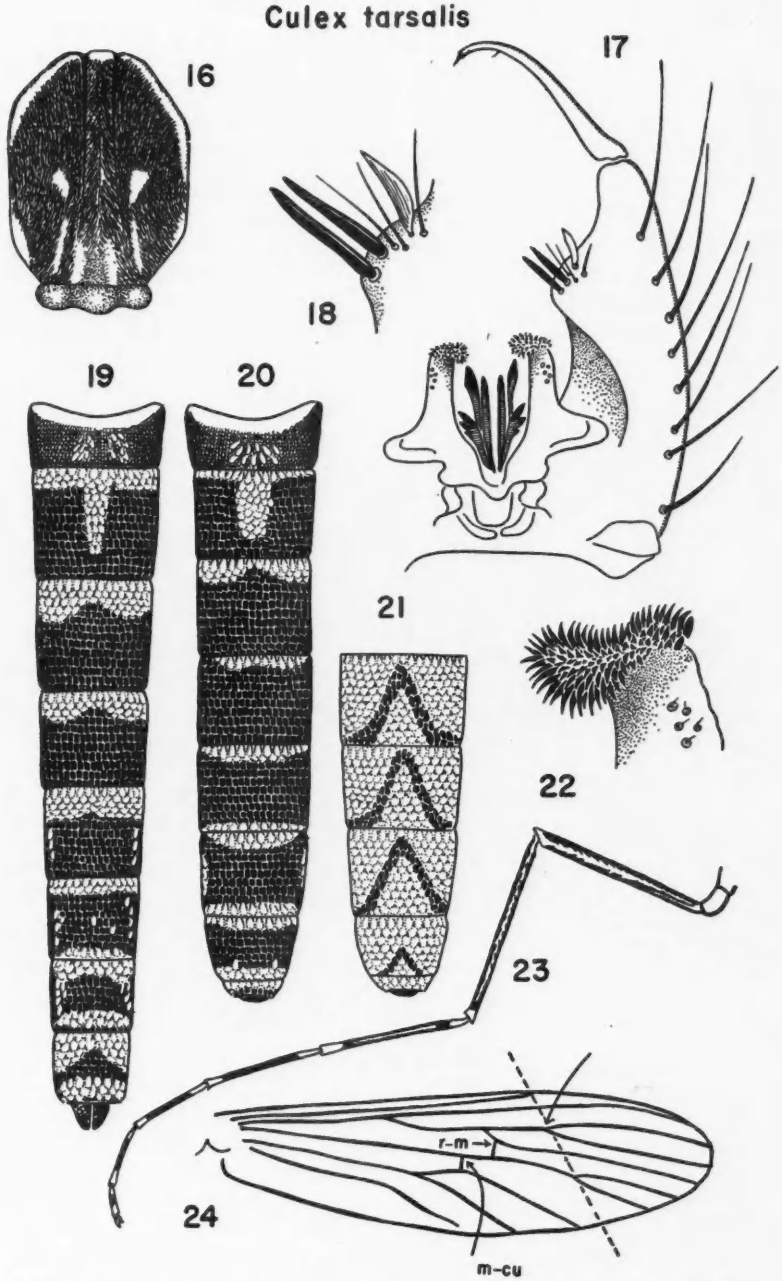
Culex tarsalis has a very wide distribution in North America. Jenkins (16) has made an extensive study of its distribution and correlates it with the geographic distribution of the virus of Western equine encephalomyelitis. Because of its vector role in the transmission of this disease, *Culex tarsalis* has been the subject of an intensive study in recent years.

Although the limits of its distribution in western Canada are fairly well known, details regarding its distribution within the limits are still largely unknown. It has been studied in Manitoba by McLintock (21) who lists nine locality records for the species based largely on light trap catches. In Saskatchewan the writer has collected the species in the following localities: Regina, Corrine, Trossachs, Estevan, Yorkton, Wynyard. The first four are districts in the open prairie, while the latter two are in the aspen grove region. Since the species is reported from the Lethbridge and Banff areas by Strickland (40) and the writer has collected specimens around Medicine Hat, it is probably present in various localities of the southwestern portion of Saskatchewan. In British Columbia, Hearle (12) found the species very generally distributed in the Fraser delta.

In Saskatchewan this species generally breeds in small temporary weedy roadside ditches, often in association with *Culiseta inornata* (Will). These ditches are filled to a depth of six inches to a foot after every rain. The water warms up quickly during the day and, although these depressions become dry in a few weeks, development proceeds at such a rate that a generation can complete its cycle.

Although it is generally accepted that the species hibernates in the adult stage, overwintering females are rarely encountered. Since the first larvae do not appear until early July in Saskatchewan, the life span of the hibernating female must extend over a period of almost eight months. In this province the problem is further complicated in that in exceptionally dry seasons the species appears to be absent. It is difficult to understand how a species that hibernates as an adult can survive under those conditions.

Since this species is found to be an important vector of encephalomyelitis of the western strain (10), an understanding of its feeding habits is important. There is however little agreement on this point. According to Hearle (12), *Culex tarsalis* is active at dusk when it makes persistent efforts to enter houses in search of blood. Moreover. he finds it to be one of the most painful biters.



FIGS. 16-24. *Culex tarsalis*. 16, mesonotum; 17, hypopygium; 18, subapical lobe; 19, abdomen of male, dorsal aspect; 20, abdomen of female, dorsal aspect; 21, abdomen of female, ventral aspect; 22, paraproct; 23, hind leg, lateral aspect; 24, wing, showing venation.

McLintock (22) reports it to bite readily during the day and often become troublesome to children. Reeves and Hammon (33) showed by means of the precipitin test that it prefers avian to mammalian blood.

Because of extensive outbreaks of Western equine encephalomyelitis in Saskatchewan the species must be considered an important one.

Genus **Culiseta** Felt

This genus is represented in our fauna by five species. Of these, *Culiseta inornata*, mainly a prairie species, is on occasion very abundant, while the other four species are largely restricted to the forested regions and are quite rare. They are rather easily separated from *Aedes* species by their blunt rounded abdomen. In three of the woodland species the wings are spotted.

Key to Females

1. Wing scales evenly spaced 2
   Wing scales on some veins densely spaced to form spots 4
2. Tarsi with narrow basal white rings; mesonotum with two broad long submedian bare stripes (Fig. 32) ..... *morsitans*
   Tarsi entirely black; mesonotum without conspicuous submedian bare stripes 3
3. Dorsum of abdomen with basal white bands widening laterally into triangular patches (Fig. 26) ..... *inornata*
   Dorsum of abdomen with basal white bands not widening laterally (Fig. 31) ..... *impatiens*
4. Hind tarsi with narrow white rings (Fig. 37); radial sector and media in front of cross veins with long closely spaced scales (Fig. 40) ..... *incidens*
   Hind tarsi with broad white rings; radial sector and media with evenly spaced scales in front of cross veins (Fig. 29) ..... *alaskaensis*

Key to Males, Based on Terminalia

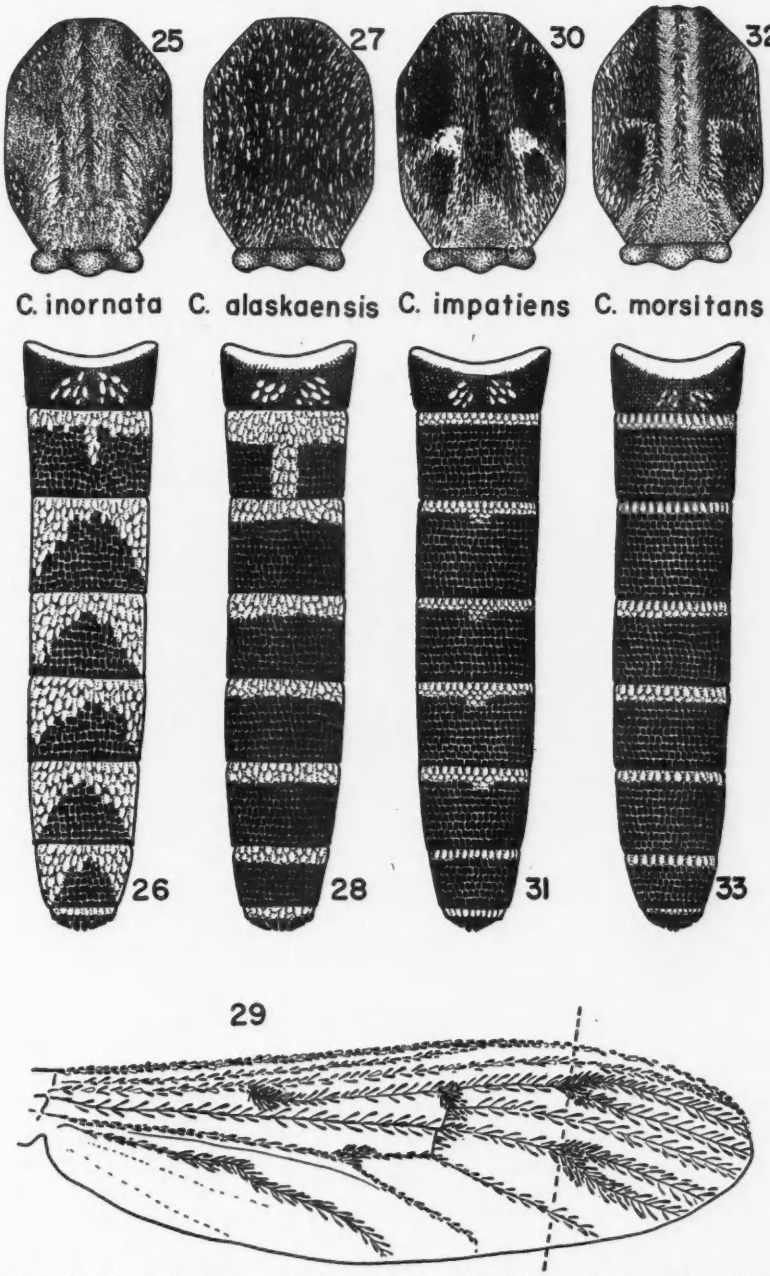
1. Apical and basal lobes present 3
   Basal lobes only present 2
2. Lobes of ninth tergite evenly rounded and approximate, with prominent spines along margin, Fig. 42 ..... *inornata*
   Lobes of ninth tergite not evenly rounded, with setae, Fig. 44 ..... *morsitans*
3. Posterior margin of eighth tergite with 30-40 spines, Fig. 45 ..... *impatiens*
   Posterior margin of eighth tergite with 3-8 spines 4
4. Posterior margin of eighth tergite generally with only three spines; apical lobe with five narrow setae, Fig. 43 ..... *alaskaensis*
   Posterior margin of eighth tergite generally with 5-8 spines; apical lobe with five setae and one or two ligulate spines, Fig. 41 ..... *incidens*

Culiseta inornata (Williston)

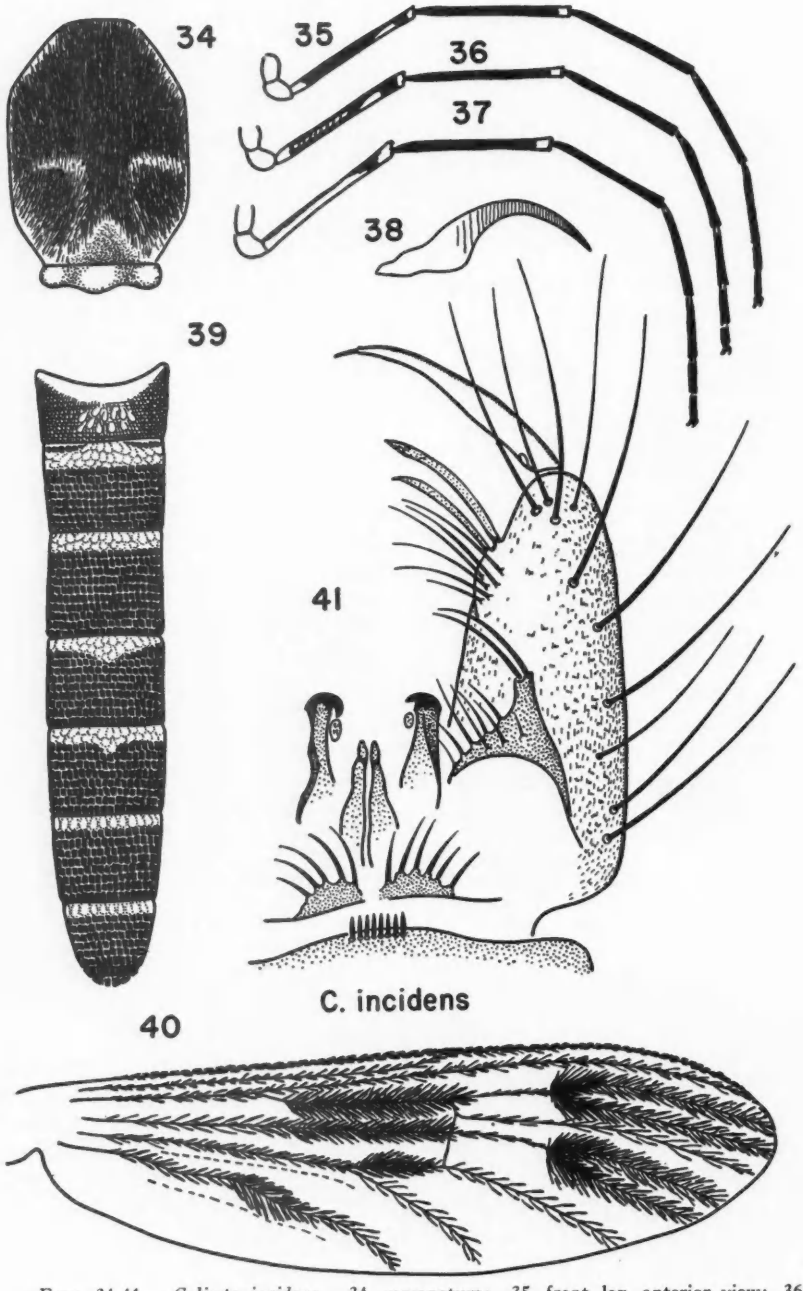
Figs. 25, 26, 42

Description

Proboscis brown with scattered white scales along ventral surface. Vertex brown with fine white scales and some black erect scales. Mesonotum (Fig. 25) with brown integument covered by golden brown hairs and some isolated white hairs, the latter may give rise to two faint white lines on the disk. Wing scales black. Legs black with scattered white scales on femora and tibiae. Abdomen (Fig. 26) with basal white bands that widen laterally into triangular patches, venter white scaled.



FIGS. 25, 26. *Culiseta inornata*. 25, mesonotum; 26, abdomen of female. FIGS. 27, 28, 29. *Culiseta alaskaensis*. 27, mesonotum; 28, abdomen of female; 29, wing, to show scale pattern. FIGS. 30, 31. *Culiseta impatiens*. 30, mesonotum; 31, abdomen of female. FIGS. 32, 33. *Culiseta morsitans*. 32, mesonotum; 33, abdomen of female.



FIGS. 34-41. *Culiseta incidens*. 34, mesonotum; 35, front leg, anterior view; 36, middle leg, anterior view; 37, hind leg, anterior view; 38, claw; 39, abdomen of female; 40, wing, to show scale pattern; 41, hypopygium.

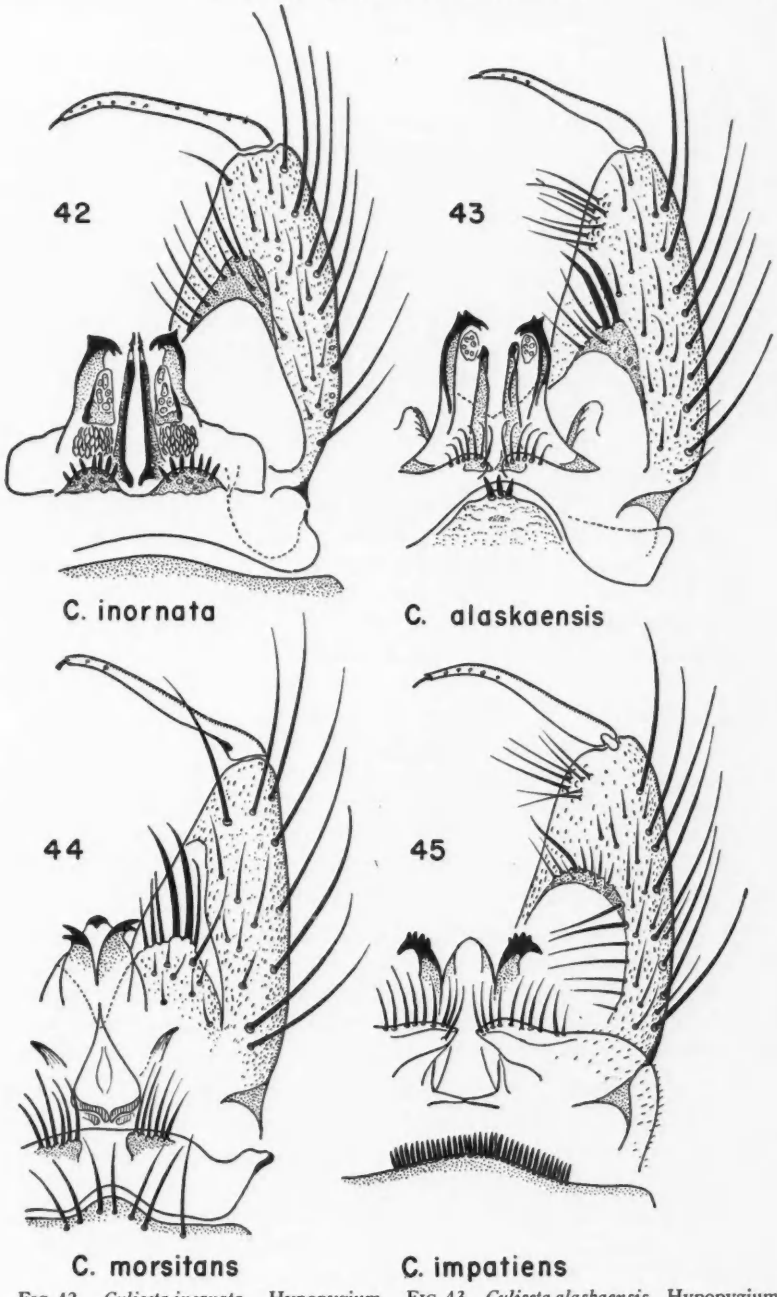


FIG. 42. *Culiseta inornata*. Hypopygium. FIG. 43. *Culiseta alaskaensis*. Hypopygium. FIG. 44. *Culiseta morsitans*. Hypopygium. FIG. 45. *Culiseta impatiens*. Hypopygium.

Hypopygium (Fig. 42). Apical lobe absent. Basal lobe with several stout bristles at apex and along margin. Paraprocts heavily sclerotized, ending apically in two or three teeth. Epiprocts heavily sclerotized, rodlike, with thin preapical chitinous area. Lobes of ninth tergite evenly rounded, provided with prominent stout spines. This last character will separate *C. inornata* from all other of our species.

Notes on Biology and Distribution

This species is found throughout the United States (Matheson (26)). It is common on the prairie lands of Manitoba, Saskatchewan, and Alberta. In Saskatchewan it is found not only in the open country, but also in the aspen grove region. It is the species that is most commonly encountered in the houses in the fall in search for a place to hibernate.

The species overwinters as females, and the larvae of the first generation may appear early in the season. In the Regina area fourth instar larvae may be found shortly after the middle of May and adults before the end of the month. During June, July, and August the species breeds extensively in shallow, grassy depressions. It shows great tolerance with respect to larval habitat. The writer has found larvae in abundance in depressions overgrown with slough grass (*Beckmannia* sp.) and wild barley (*Hordeum jubatum* L.); in stagnant highly alkaline (pH 8.8) water where the vegetation was mainly sedges (*Carex* sp.) and bulrush (*Scirpus* sp.) and in clear water of depressions with clayey bottom (Fig. 158).

There is much overlapping of generations for, at any habitat, eggs, various instar larvae, pupae, and adults may be found at the same time. Unlike most of our other species of mosquitoes, the males of *Culiseta inornata* do not swarm. Mating normally takes place on vegetation a few inches above the surface of the water. Since the species readily mates in captivity, laboratory colonies are established with ease (Owen (29)).

This species is not annoying to man, but on occasion may severely attack stock. It is looked upon as a potential vector of Western equine encephalomyelitis and St. Louis encephalitis. During the 1941 epidemic of equine encephalomyelitis in Saskatchewan, it formed an important part of the mosquito fauna.

Recently Wilkins and Breland (48) have shown that in the southern part of the United States the species may pass the summer in the egg stage. If it were found that it could also pass the northern winter in the egg stage, a perplexing problem of its life cycle would be solved. In some seasons the species builds up large populations, while in other seasons it seems to be absent. It is difficult to see how a species that it supposed to hibernate exclusively as an adult can survive under such adverse conditions.

Culiseta alaskensis (Ludlow)

Figs. 27, 28, 29, 43

Description

Proboscis brown. Vertex brown with numerous white appressed scales and numerous erect narrow and forked brown scales. Mesonotum (Fig. 27) black with brown and white scales evenly intermingled. Wing scales black forming spots at base of *Rs*, over cross veins, at fork of R_{2+3}, at fork of *M*, and over base of cubital fork (Fig. 29). Legs with broad white rings on tarsi. Abdomen (Fig. 28) black with basal white rings, the one on segment two extending backwards as a median bar.

Hypopygium (Fig. 43). Apical lobe with fine setae. Basal lobe with two very prominent spines and several narrow spines. Lobes of ninth tergite lunar shaped with five setae along margin. Median lobe of eighth tergite with three stout spines.

Notes on Biology and Distribution

This species is found in Alaska south through Western Canada and along the mountainous areas as far as Colorado. It has also been reported from Labrador (11), Quebec (18), and Manitoba (14). It has a wide distribution in Europe and Asia (Natvig (28)). In Saskatchewan the writer has found the species at Waskesiu in late May.

The writer has not been able to locate its breeding places. Jenkins (15) made extensive collections of the larvae in Alaska and the following quotation is taken from his paper (p. 146):

> "They were fairly abundant locally in *Carex* swamps, and in the *Carex* and grass at the edge of small pools and ponds. They were abundant in the small pools among *Carex* and *Myrica* in coastal marshes. Larvae were collected in a bog, in a permanent pond containing duckweed and in a small pool containing decaying logs. They were found in shallow or deep pools and marshes of various sizes. The breeding places were usually open and unshaded. All instars of larvae were found from May 11 to July 10."

At Churchill, Man., Hocking *et al.* (14) found fourth-instar larvae on July 21.

Adults may be encountered in northern coniferous areas in early spring. In Alaska, Jenkins (15, p. 147) found that

> "overwintering females were common and bit readily from the latter part of April until the middle of June. . . . . Biting was more common on the upper part of the body, especially on the darker areas of the skin and at the hair line. The females were most aggressive during the long crepuscular periods, although they would bite throughout the day."

Hadwen and Palmer (9) state that this species annoys man and reindeer in Alaska in early spring. Further observations on feeding habits have been made by Jenkins and Knight (18) in Quebec. According to Hearle (12, p. 57) this species is of little importance in the Lower Fraser Valley of British Columbia "as it is timid in attack and a poor biter".

Culiseta impatiens (Walker)

Figs. 30, 31, 45

Description

Proboscis brown. Vertex brown covered with fine white scales. Mesonotum (Fig. 30) dark brown with golden brown and white scales, the white scales forming two distinct spots on the disk and two white lines extending from these spots back to the scutellum; white scales around antescutellar area, above the wing bases, and near anterior margin. Wing scales black, longer and denser at base of radial sector, at fork of R_{2+3} and at fork of media. Legs dark scaled. Abdomen (Fig. 31) with narrow basal white bands and short median extensions.

Hypopygium (Fig. 45). Apical lobe with prominent setae; basal lobe large, rounded, and narrow with many long setae along lower margin, followed by small setae and one large seta at apex. Paraprocts heavily sclerotized apically, with four or five teeth. Lobes of ninth tergite large with numerous setae. Eighth tergite with 30 to 40 stout spines along margin. The latter character will separate the species from all of our other species.

Notes on Biology and Distribution

This species is widespread in the coniferous forest areas of northern North America. In the mountain areas of the west it extends south through Montana, Wyoming, into Utah. According to Matheson, it is found from coast to coast. The larvae are said to prefer dark permanent forest pools, although Hearle and Jenkins have also collected them from muddy water in road ruts. In Saskatchewan the writer has collected adults at Waskesiu in late May, but he has not found larvae.

Observations on the feeding habits of the adult have been made by Hearle (12), by Jenkins (15), and by Jenkins and Knight (18). Hearle states that the species is timid and hesitating in its attack, but that on emergence from hibernation quarters in the spring it may bite fiercely for a few days. Jenkins and Knight found it to bite at any time of the day, but not in strong wind. According to Rees (32) the species feeds normally at dusk and at times is vicious in its attack.

The adult hibernates in a sheltered place and may be found on the wing during the first warm days of spring. It is rare in Saskatchewan.

Culiseta morsitans (Theobald)

Figs. 32, 33, 44

Description

Proboscis with brown scales. Vertex with fine appressed white scales and numerous brown erect forked scales, the latter giving the head a bushy appearance. Mesonotum (Fig. 32) dark brown, with two bare stripes variable in width; vestiture of narrow golden brown scales and some white scales, the latter giving an indication of two white lines on the disk. Wing scales black,

with no indication of spots. Legs dark brown with some white scales at tips of tibiae, at both ends of basal three tarsal segments, and along outer surface of tibiae. The white markings are more distinct in females than in males. Abdomen (Fig. 33) dark brown with narrow, even, white basal bands.

Hypopygium (Fig. 44). Apical lobe absent; basal lobe large, rounded, with spines along margin, two of which are larger than the remainder. Paraprocts sclerotized apically, ending in two fine teeth. Lobes of ninth tergite small, rather far apart and each with a row of marginal setae. Lobe of eighth tergite with long setae. In the latter respect the North American form differs from the European as described and figured by Natvig (28). This author (p. 174) states that the tip of the eighth tergite has three to seven spines. Judging by his figure, these spines are prominent and could not be overlooked.

Notes on Biology and Distribution

This species is found in Alaska, it is widespread in Canada and extends south into New Jersey, Wisconsin, Indiana, Illinois, and Colorado. If we are correct in assuming that the North American and the European forms are the same species, then its distribution also extends through Northern Europe as far east as the Urals and south into Roumania and the Ukraine (28, p. 178).

In Saskatchewan the writer has collected the species at Waskesiu in early July. He has also seen specimens from Indian Head collected by E. Hearle in late July. Oddly enough the species is not reported from Manitoba by McLintock, nor from Alberta by Strickland, nor from the Lower Fraser Valley of British Columbia. Dyar (3) however, gives its distribution in Canada as Ontario, Alberta, British Columbia, and Yukon Territory. Observations on the larval habitat have been made by Jenkins and Knight (19) and by Ross (36) on this continent and in Europe by Wesenberg-Lund, Marshall, Martin, and Peus (references from Natvig (28, p. 179)). At the southern end of James Bay, Ontario, Jenkins and Knight found larvae most frequently in habitats containing sedges. Ross collected larvae in cool shaded pools in a tamarack bog. In Europe *C. morsitans* overwinters as a full-grown larva under ice.

It is generally stated that the adult does not attack man.

Culiseta incidens (Thomson)

Figs. 34-41

Description

Proboscis with brown scales. Vertex with numerous narrow white and brown scales and some erect brown forked scales. Mesonotum (Fig. 34) with dark brown and scattered white scales, the latter may form spots near the center of the disk, and two white lines from these spots extending posteriorly to the antescutellar area. Wing scales (Fig. 40) dark brown arranged in patches as follows: along *Rs* from near its base to the cross veins, at base of fork of R_{2+3}, along posterior third of *M*, at base of fork of *M*, near base of *Cu*, and near middle of 2*A*. Front leg (Fig. 35) black; base, apex, and preapical

patch of femur, apex of tibia, base of second and third tarsal segments white scaled. Middle leg (Fig. 36) similar to front leg, with additional row of white scales on femur and basal white rings on tarsal segments 2-4. Hind leg (Fig. 37) with under surface of femur white. The white bands on the tarsal segments are very narrow and frequently obscure. Abdomen (Fig. 39) with narrow basal white bands.

Hypopygium (Fig. 41). Apical lobe with one or two broad spines and several setae; basal lobe with narrow projection ending in two long spines. Paraprocts sclerotized apically with three teeth at tip. Lobes of ninth tergite approximate, sclerotized, with five or sixpines along margin. Median lobe of eighth tergite with blunt spines (average number eight).

Notes on Biology and Distribution

According to Matheson (26) *Culiseta incidens* occurs west of the Rocky Mountains from Alaska to Southern California. Although the Rocky Mountains have been considered the limit of its easterly distribution, Mail (personal communication) has collected the species in South Dakota. The Saskatchewan records are based on a series of larvae collected by Dr. K. M. King at Waskesiu on July 2–4, 1929, and identified by the late Eric Hearle as *C. incidens*. The writer has not been able to check the identification.

Extensive observations on the biology of this species have been made by Hearle in British Columbia. It is one of the most common and widespread mosquitoes in the Lower Fraser Valley where it breeds mainly in rain barrels. The adult female hibernates. There are several generations. According to Hearle (12) the female is timid and rarely attacks man. However, Freeborn and Bohart (4) find that in California one strain will avidly attack man.

Genus **Aedes** Meigen

Most Canadian species of mosquitoes belong to the genus *Aedes*. Twinn (42) records a total of 39 species of this genus for Canada. Twenty-five of these species are reported here for Saskatchewan. When we consider the economic importance of our mosquitoes, the genus *Aedes* stands out and all the other genera fade almost into insignificance. On our prairies, in our park lands, in the forested zones, and in the arctic the picture is similar. The hordes of attacking mosquitoes belong to *Aedes*.

In the description of the species, emphasis has been on the vestiture of the mesonotum and abdomen, and on the male terminalia. Vockeroth (45) has used the tarsal claw as an aid to separating closely related species of five of our band-legged forms. The expression claw ratio used in this paper refers to the length of the main tooth in relation to the length of the basal portion of the claw. In most cases mounts of the claws of only a half a dozen individuals were made. Hence the applicability of the claw ratio needs further study. It is, however, useful in separating *A. excrucians* (Walker) from two other common band-legged species of this area: namely *A. fitchii* (F. & Y.) and *A. increpitus* Dyar.

Key to Females

1. Tarsal segments with white rings 2
   Tarsal segments without white rings 12
2. White rings basal and apical 3
   White rings basal only 5
3. Wings with black scales ..... *canadensis*
   Wings with black and white scales, intermingled 4
4. R_{4+5} black scaled, Fig. 71 ..... *dorsalis*
   R_{4+5} largely white scaled, Fig. 73 ..... *campestris*
5. White rings very narrow ..... *vexans*
   White rings broad 6
6. Large yellowish species, abdomen dorsally entirely yellow or with yellow and black scales intermingled 7
   Medium dark species, abdomen black with distinct basal white bands 8
7. Abdomen yellow dorsally, with two dark spots on each segment, Fig. 87 ..... *flavescens*
   Abdomen with black and yellow scales intermingled and faint indications of basal bands, Fig. 91 ..... *riparius*
8. Proboscis with white ring; abdomen black with conspicuous white T-shaped markings, Figs. 85, 86 ..... *nigromaculis*
   Proboscis not ringed with white 9
9. Mesonotum with a broad chocolate-colored median stripe, sides whitish, Fig. 49; abdominal white bands generally not reaching the sides, band on second segment T-shaped, Fig. 50; wings with black and white scales ..... *fitchii*
   Not as above 10
10. Mesonotum with white scales on the disk forming spots or stripes 11
    Mesonotum golden brown, with faint indication of a median brown stripe, but without white spots or lines, Fig. 52 ..... *stimulans*
11. Mesonotum with white scales forming lines, Fig. 46; basal band on abdominal segment two not reaching the sides; claw ratio 1 : 1, Fig. 48 ..... *increpitus*
    Mesonotum with white scales forming spots, Fig. 55; basal band on abdominal segment two reaching the sides; claw ratio 2 : 1, Fig. 57 ..... *excrucians*
12. Mesonotum with distinct dark stripe or stripes 13
    Mesonotum uniformly colored, rarely with faint median dark stripes 21
13. Mesonotum with single median dark stripe 14
    Mesonotum with paired dark stripes 17
14. Median brown stripe widening posteriorly, remainder of disk and margin around antescutellar area with silvery white scales, Fig. 126; abdominal segments black with rounded patches of white laterally on segments four to seven, Fig. 125 ..... *triseriatus*
    Median brown stripe not widening posteriorly, disk without silvery white scales 15
15. Wings entirely brown scaled; abdomen black with basal white rings 16
    Wings with some veins entirely white scaled, Fig. 146; abdomen mainly or entirely white scaled ..... *spencerii*
16. Median brown stripe narrowing posteriorly, white scales on disk shading laterally through yellow into brown, Fig. 129; abdominal white bands narrow, widening slightly at the sides, Fig. 128 ..... *sticticus*
    Median brown stripe of uniform width, scales on disk whitish or yellowish, not shading into brown laterally, Fig. 122; abdominal bands widen into triangular patches laterally, Fig. 121 ..... *punctor*
17. Mesonotal stripes broad, black or dark brown, separated by a narrow line of yellowish scales, Fig. 99 ..... *pionips*
    Mesonotal stripes narrow, widely separated 18
18. Mesonotal stripes divided by yellowish white bands 19
    Mesonotal stripes divided by light brown bands 20
19. Sides of mesonotum yellowish, basal abdominal bands narrow, widening laterally ..... *communis*
    Sides of mesonotum silvery white; abdominal segments with lateral triangular spots ..... *diantaeus*
20. Mesonotum with a narrow bare median black line, Fig. 142 ..... *pullatus*
    Mesonotum without a narrow bare median black line, Fig. 107 ..... *impiger*
21. Mesonotum with brown and white scales intermingled, imparting a "frosted" appearance to it, Fig. 111 ..... *trichurus*
    Mesonotum without intermingled white and brown scales 22
22. Mesonotum dark brown medially with two faint blackish stripes, sides shading from golden brown to white, Fig. 104 ..... *cataphylla*
    Mesonotum uniformly brown 23
23. Abdomen with broad basal white bands 24
    Abdomen without basal white bands, Fig. 136 ..... *cinereus*
24. Venter of abdomen entirely white scaled ..... *intrudens*
    Venter of abdomen white scaled but with posterior margins of segments black ..... *nearcticus*

KEY TO MALES BASED ON TERMINALIA

1. Dististyle inserted before the apex of basistyle, Fig. 138 ..... *cinereus*
   Dististyle inserted at apex of basistyle 2
2. Basistyle without basal and apical lobes 3
   Basal or apical lobe, or both, present 4
3. Dististyle furcate apically, Fig. 79 ..... *vexans*
   Dististyle not furcate apically, Fig. 127 ..... *triseriatus*
4. Basal and apical lobes present 6
   Only one lobe present 5
5. Apical lobe only, a setagerous area in place of basal lobe, Fig. 64 ..... *excrucians*
   Apical lobe absent, basal lobe present, Figs. 83, 84 ..... *nigromaculis*
6. Dorsal surface of apical lobe bare, or nearly bare 7
   Dorsal surface of apical lobe with hairs or setae 10
7. Basistyle with a group of long setae arising near the base of the apical lobe, Fig. 105 8
   Basistyle without a group of long setae arising near the base of the apical lobe 9
8. Claspette evenly curved; filament as long as stem, enlarged, with outer edge parallel to inner edge for some distance, Fig. 106; lobes of ninth tergite conical, with three to seven spines, Fig. 109*a*; basal lobe with a row of setae, the first one considerably longer than the others, Fig. 110*b* ..... *cataphylla*
   Claspette bent elbow-like; filament conspicuously enlarged near base and tapering gradually toward apex, Fig. 108; lobes of ninth tergite low, each with seven to nine spines, Fig. 109*b*; basal lobe with a row of setae, the first one spinelike and only slightly larger than the others, Fig. 110*a* ..... *impiger*
9. Stem of claspette evenly rounded, Fig. 135; basal lobe with one small spine and several setae, Fig. 134 ..... *nearcticus*
   Stem of claspette elbowed, Fig. 143; basal lobe with three prominent spines, Fig. 144 ..... *pullatus*
10. Apical lobe with short retrorse setae 11
    Apical lobe with long setae 13
11. Basal lobe constricted near base; stem of claspette straight 12
    Basal lobe not constricted near base, Fig. 123; stem of claspette markedly curved, Fig. 124 ..... *punctor*
12. Basal lobe with a spine and a dense row of long setae, Fig. 131; filament angularly enlarged near base ..... *sticticus*
    Basal lobe with a spine and a group of small setae, Fig. 152; filament not angularly enlarged near base, Fig. 147 ..... *spencerii*
13. Basal lobe a double structure, Fig. 113; claspette very long curved and ribbed apically, Figs. 112, 114 ..... *trichurus*
    Basal lobe a single structure 14
14. Basal lobe with numerous setae but without a distinct spine 15
    Basal lobe with one or more spines 16
15. Filament of claspette with toothlike projection on the outer margin, Fig. 58 ..... *increpitus*
    Filament of claspette without a toothlike projection 24
16. Basal lobe with numerous setae and at least one spine 18
    Basal lobe with a few fine setae and three prominent spines 17
17. Basistyle with a tuft of setae near the apex, Fig. 117; filament of claspette foot-shaped, Fig. 116 ..... *intrudens*
    Basistyle with a row of long hairs on the ventral side, Fig. 141; filament of claspette sickle-shaped, Fig. 140 ..... *diantaeus*
18. Basal lobe with setae and one spine 19
    Basal lobe with setae and two spines, Figs. 75, 77 ..... *dorsalis*
19. Filament of claspette angularly expanded on outer margin 20
    Filament of claspette with outer margin rounded 23
20. Filament almost as long as stem, Fig. 60 ..... *stimulans*
    Filament much shorter than stem 21
21. Filament angularly expanded on inner and outer margin, Fig. 62 ..... *fitchii*
    Filament angularly expanded on outer margin only 22
22. Posterior surface of basal lobe convex, Fig. 89 ..... *flavescens*
    Posterior surface of basal lobe concave, Fig. 93 ..... *riparius*
23. Posterior surface of basal lobe convex, setae along margin, slightly curved, Fig. 101 ..... *pionips*
    Posterior surface of basal lobe concave; setae along margin markedly curved, Fig. 97 ..... *communis*
24. Filament of claspette narrow, awl-shaped; apical lobe large, Fig. 120 ..... *canadensis*
    Filament of claspette expanded near base; apical lobes small, Fig. 78 ..... *campestris*

Aedes increpitus Dyar

Figs. 46, 47, 48, 58, 59

Description

Proboscis dark brown. Vertex with numerous narrow white scales and erect forked brown scales. Mesonotum (Fig. 46) golden brown with white scales near the margin and in the form of longitudinal white lines on the disk. Wing scales black with white scales as a patch over base of costa and along subcosta. Legs dark scaled with numerous white scales on femora and tibiae; tarsal segments banded basally with white, the bands narrow on the front and middle legs but broad on hind legs. Tarsal claw ratio (Fig. 48) 1 : 1. Abdomen (Fig. 47) with white basal bands, band on segment two does not reach the sides, other bands widen at the sides.

Hypopygium (Figs. 58, 59). Basistyle long and narrow. Apical lobe small with a few setae; basal lobe (Fig. 59) with small setae but without a spine, and extending far up toward the base of the apical lobe. Filament of claspette angularly expanded near the middle.

Notes on Biology and Distribution

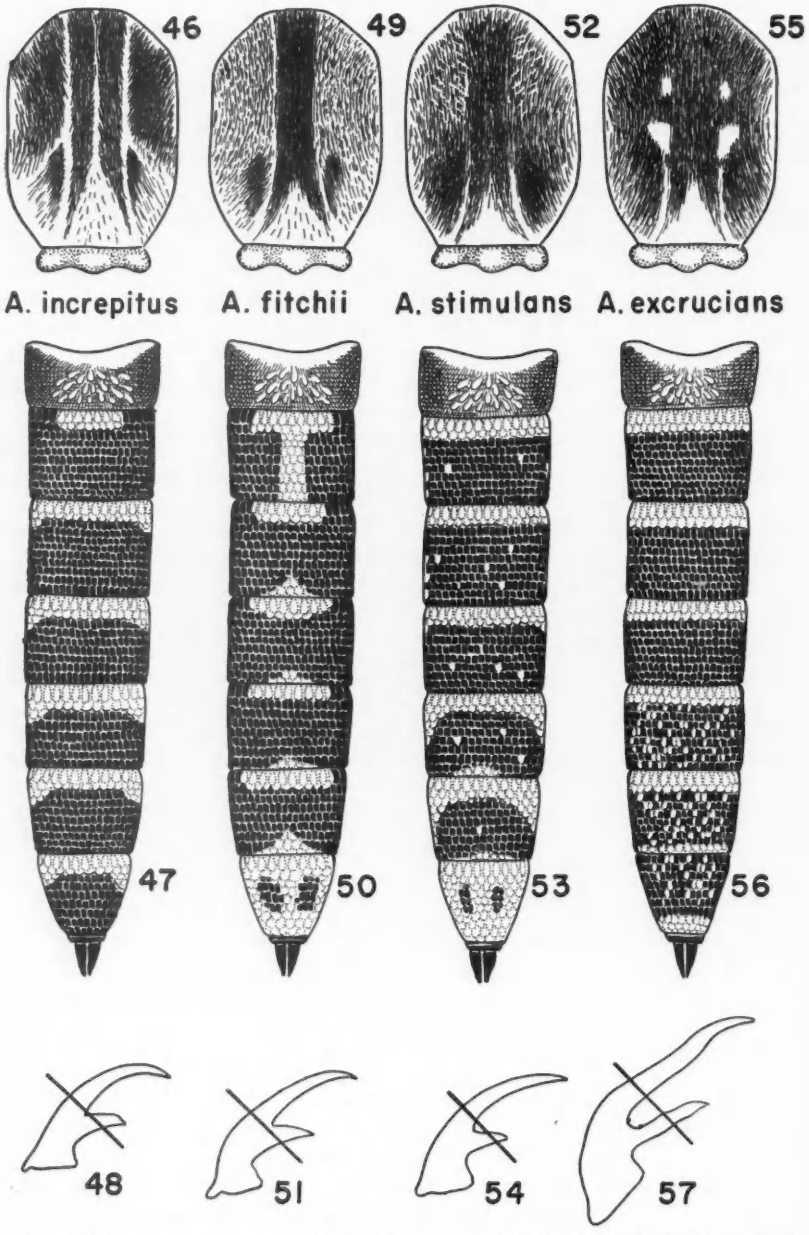
This species is found in the coastal states from British Columbia to Mexico and inland across the Rockies to the plains. It is rare and localized in Saskatchewan. The writer has reared a long series of adults from larvae collected in a small slow flowing stream in a wooded valley near Regina (Boggy Creek) (Fig. 157). This little stream is flowing in early spring and after rains. During most of the summer the water is stationary. However, it does not become stagnant because of the continually low temperature of the water. A second collection of larvae was made in a shallow woodland pool at Borden. It is an early species. The larvae may be collected at the beginning of May and the adults appear toward the end of May. They may be on the wing throughout June. The species feeds readily in the woods in daylight, but does not appear to leave its habitat.

Aedes fitchii (Felt and Young)

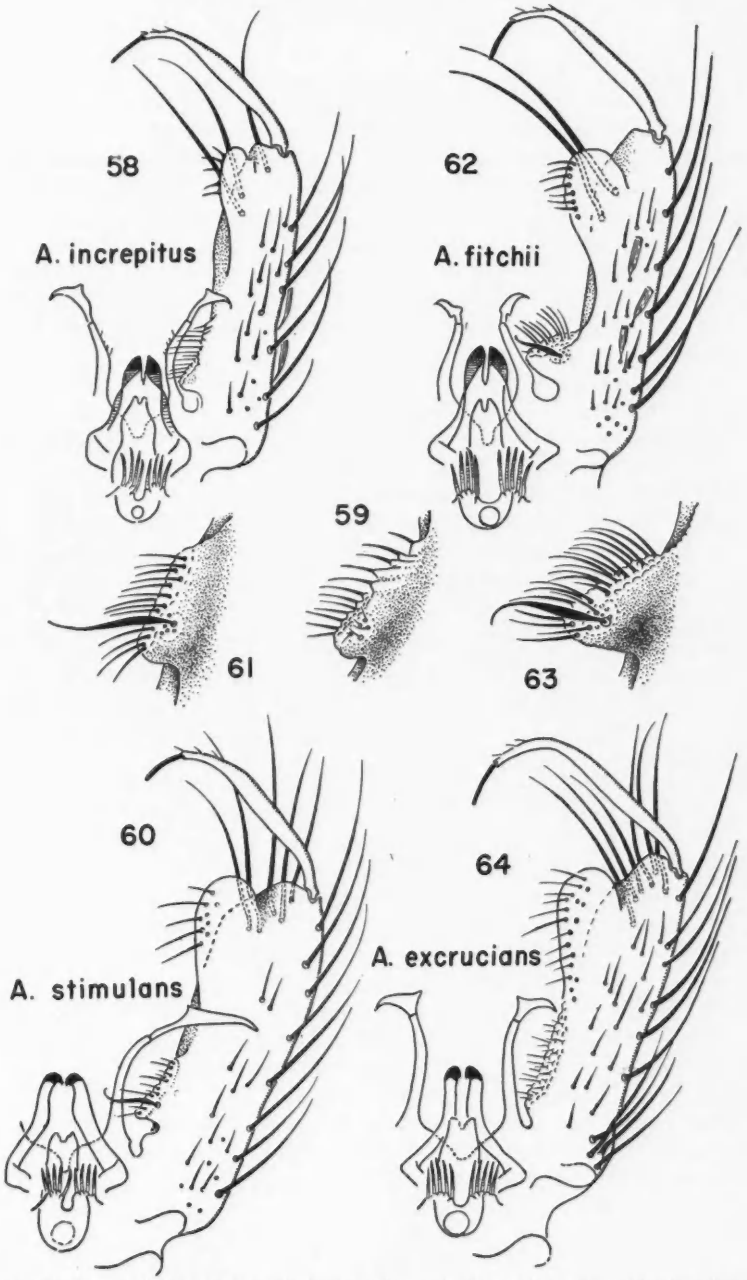
Figs. 49, 50, 51, 62, 63

Description

Proboscis brownish-black. Vertex with fine white and brown scales and erect brown forked scales; cheeks with appressed white scales and a patch of brown scales in line with the middle of the eye. Mesonotum (Fig. 49) with broad median band of chocolate brown scales, remainder mainly white. Wings with black and white scales intermingled on all veins. Legs with intermingled black and white scales on femora and tibiae and metatarsi; tarsal segments with broad basal white bands. Claw ratio 0.8 : 1.2. Abdomen (Fig. 50) with basal white bands not reaching the sides; white scales on segment two tending to form a median bar; segment seven nearly



FIGS. 46-48. *Aedes increpitus*. 46, mesonotum; 47, abdomen of female; 48, claw. FIGS. 49-51. *Aedes fitchii*. 49, mesonotum; 50, abdomen of female, 51, claw. FIGS. 52-54. *Aedes stimulans*. 52, mesonotum; 53, abdomen of female; 54, claw. FIGS. 55-57. *Aedes excrucians*. 55, mesonotum; 56, abdomen of female; 57, claw.



FIGS. 58, 59. *Aedes increpitus*. 58, hypopygium; 59, basal lobe. FIGS. 60, 61. *Aedes stimulans*. 60, hypopygium; 61, basal lobe. FIGS. 62, 63. *Aedes fitchii*. 62, hypopygium; 63, basal lobe. FIG. 64. *Aedes excrucians*. Hypopygium.

all white. Hypopygium (Figs. 62, 63). Apical lobe prominent, with setae. Basal lobe (Fig. 63) triangular in shape with numerous long setae and one curved spine. Filament of claspette short, angularly expanded near the base.

Notes on Biology and Distribution

A. fitchii is a common mosquito of the forested areas of North America. In Saskatchewan it is frequently encountered in the isolated poplar bluffs of the prairie; it is the most abundant species in the aspen grove region in the early season; it is also fairly common in the coniferous forested areas. The locality records for this species are too numerous to report. The writer has a very large collection of all stages from Saskatchewan as far south as Regina and as far north as Lac la Ronge. The species is also common in the Cypress Hills area.

A. fitchii prefers woodland pools for breeding. It is our most common species in the transition zone from prairie to forest. The larvae are found generally throughout May. In the lower limits of the aspen grove zone the adults are on the wing in June, while in our coniferous forest areas they are common in early July. There is only one generation.

Aedes stimulans (Walker)

Figs. 52, 53, 54, 60, 61

Description

Proboscis black with scattered white scales. Vertex with fine white and brown scales evenly distributed giving it a fuscus appearance; cheeks with flat appressed scales and a small black patch behind the eye. Mesonotum (Fig. 52) golden brown in color with faint indication of a median stripe. Wing scales predominantly black. Legs with white and black scales on femora and tibiae; hind tarsal segments with broad basal white rings; bands on fore and mid tarsi narrow. Claw ratio 1.2 : 0.8. Abdomen (Fig. 53) with basal white bands which widen markedly at the sides on segments five and six; segment seven almost completely white scaled.

Hypopygium (Fig. 60). Apical lobe large, with a few setae. Basal lobe a quadrately expanded area with fine setae and one curved marginal spine (Fig. 61). Filament of claspette long, narrow basally, sharply expanding into an angular projection and then narrowing gradually to the tip.

The adult of *A. stimulans* closely resembles that of *A. fitchii*. The presence of scattered white scales on the proboscis; the absence of a chocolate colored median mesonotal stripe, and the predominance of black scales on the wing characterize the former.

The hypopygium closely resembles that of *A. increpitus*. The species can be distinguished by the shape of the filament of the claspette and the presence of a spine on the basal lobe.

Notes on Biology and Distribution

This species has a wide distribution in the northern United States and Canada. According to Matheson (26) it is the most abundant and annoying

woodland species of the northeastern states. In Saskatchewan it appears to be rare and localized. The writer has a few female specimens from Waskesiu, identified by Dr. Matheson. One adult male collected by the writer at Dubuc on May 24, 1944, is definitely this species. Since McLintock does not report the species from the Greater Winnipeg area, the distribution in Manitoba is probably equally limited.

According to Matheson (26, p. 150) *A. stimulans* in New York and other eastern states "is a vicious biter and occurs in enormous numbers, rendering life almost unendurable. It is a common invader of urban communities, villages and parks". This is an early species. Larvae are found during the later part of May and adults from late May to early July.

Aedes excrucians (Walker)

Figs. 55, 56, 57, 64

Description

Proboscis with brown and white scales, the latter predominating. Vertex with fine white and brown scales, the former concentrated near margin of eyes and in a narrow median line; forked erect brown hairs numerous; cheeks with appressed white scales and a small patch of black ones. Mesonotum golden brown with white scales forming spots and lines on the disk. The general pattern is illustrated in Fig. 55. Wing scales mainly brown, but white scales on anterior veins very numerous. Legs with brown and white scales; lower surface of femora, tibiae, and metatarsi almost entirely white; tarsal segments with broad basal white rings. Claw ratio almost 2 : 1. The claw (Fig. 57) is characteristic in shape and affords a convenient method of separating this species from the other members of the *stimulans* group. Abdomen (Fig. 56) with basal white bands and with numerous white scales amongst the brown scales on segments five, six, and seven.

Hypopygium (Fig. 64). Basal lobe a flat rugose area extending from the base of the side piece to the apical lobe. The setae are small, borne on small tubercles. The shape of the basal lobe makes identification of the male easy.

Notes on Biology and Distribution

This species has an extremely wide geographic distribution. In North America it is found in the northern States and generally throughout most of Canada. Its distribution extends from our plains areas through the aspen grove and forested regions into the tundra. It has also a wide distribution in Europe and Asia (28). In Saskatchewan the species, although frequently encountered, is never found in large numbers. This is true in the case of larvae as well as adults. Our records include: Regina, Dundurn, Arran, Wadena, Borden, Waldheim, Fish Creek, White Fox, Waskesiu, Lac la Ronge.

The larvae are frequently collected in small woodland pools in early May. There is one generation in a season. Since the adults are long lived, occasional specimens may be encountered in midsummer.

Aedes dorsalis (Meigen)

Figs. 65, 66, 67, 71, 72, 75, 77

Description

This species is frequently difficult to separate from *Aedes campestris* Dyar and Knab.

Proboscis black, with few white scales. Vertex with numerous fine white and brown scales; erect forked scales largely yellowish white; cheeks with appressed white scales and a patch of brown scales. Mesonotum (Fig. 65) whitish with broad median rust colored stripe that frequently ends posteriorly in three narrow processes. Wings with black and white scales, the latter predominate on the anterior veins, R_{4+5} dark scaled (Fig. 71). Legs largely white scaled on femora and tibiae; tarsal segments with white bands at both ends. Claw ratio 1.2 : 0.8, main tooth (Fig. 72) in line with base, second tooth short and divergent. Abdomen of the female (Fig. 67) white scaled with black patches that progressively get smaller distally. Abdomen of the male (Fig. 66) black scaled with white T-shaped markings on segments two to seven.

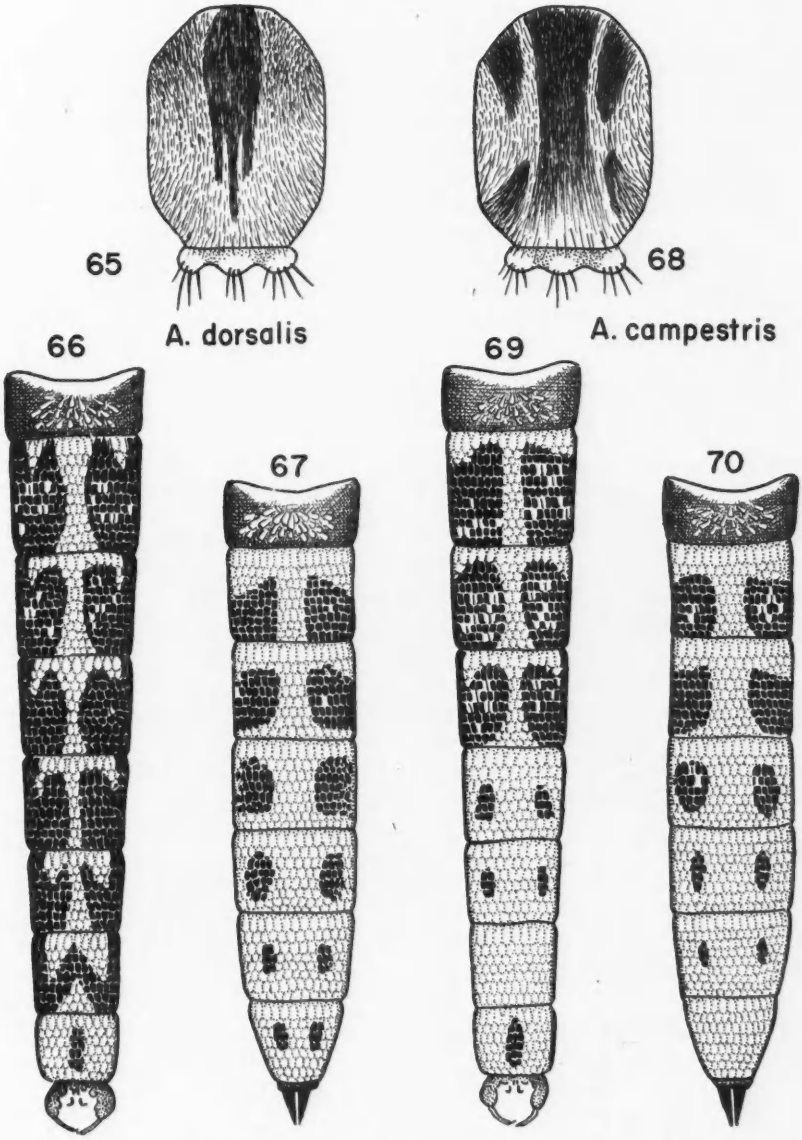
Hypopygium (Fig. 77). Apical lobe small, with setae. Basal lobe semi-detached with two spines and numerous setae (Fig. 75).

Notes on Biology and Distribution

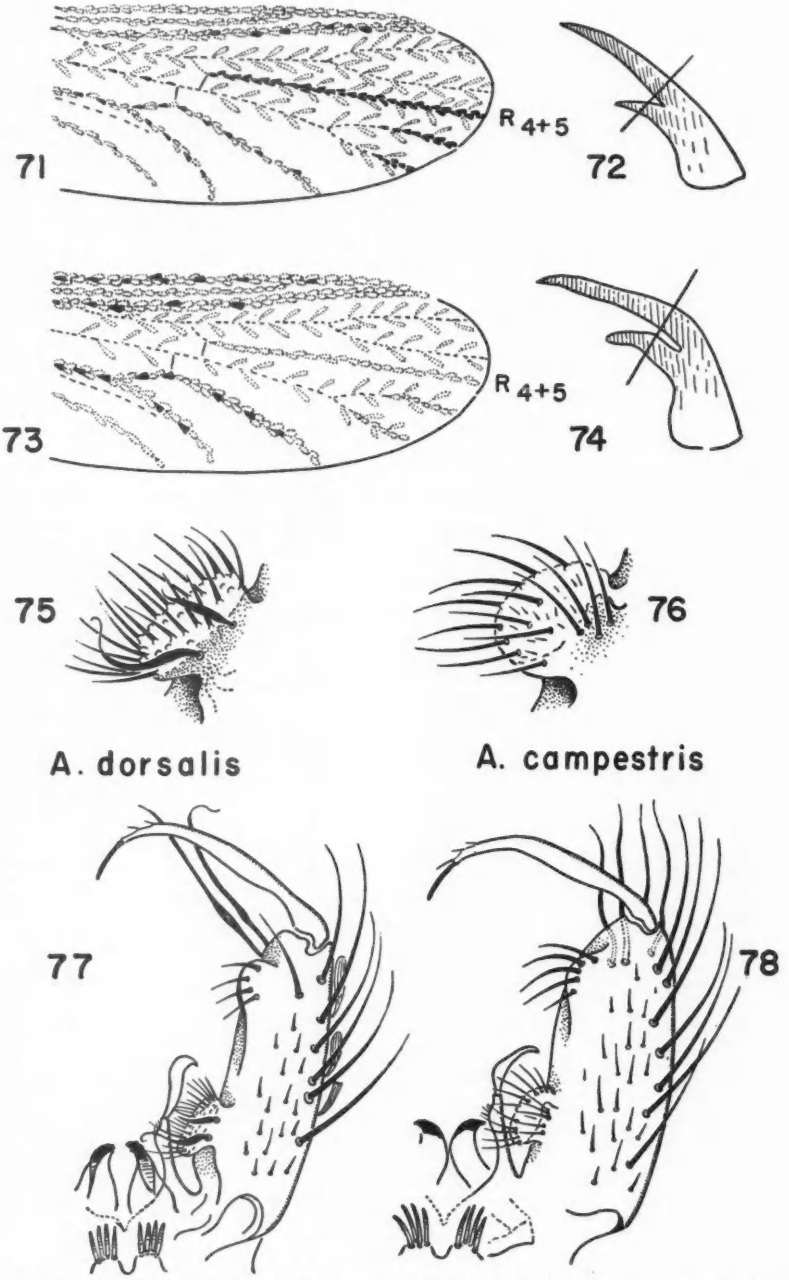
A. dorsalis is a common mosquito in the Western United States, Canada, Asia, and Europe. In Saskatchewan it is one of our common prairie mosquitoes. Our locality records include Kisbey, Yellow Grass, Regina, Yorkton, Dafoe, Dundurn, Nipawin.

The larvae of *A. dorsalis* generally appear in early July and the adults are on the wing during the latter part of July and during August. The larval habitat is usually a shallow weedy pool where the water is alkaline and rich in organic matter (Fig. 153). At the northern outskirts of the city of Regina a large dumping ground is an ideal breeding place for this species and for *A. campestris* D. and K. (Fig. 156). About eight acres of uneven land is heavily overgrown with wild barley (*Hordeum jubatum*) and alkali grass (*Puccinellia* sp.). The water that accumulates here has drained through decaying hay, straw, and manure and is consequently rich in organic matter. The above named species breed here in tremendous numbers and become a serious pest to the city dwellers.

The species overwinters in the egg stage and the eggs are said to remain viable for several years. That would explain the rarity or absence of this species on our plains in seasons of drought and the great population with the return of a wet season. If conditions are favorable one brood emerges in July and a second brood at the beginning of September.



FIGS. 65-67. *Aedes dorsalis*. 65, mesonotum; 66, abdomen of male; 67, abdomen of female. FIGS. 68-70. *Aedes campestris*. 68, mesonotum; 69, abdomen of male; 70, abdomen of female.



FIGS. 71, 72, 75, 77. *Aedes dorsalis*. 71, wing; 72, claw; 75, basal lobe; 77, hypopygium.
Figs. 73, 74, 76, 78. *Aedes campestris*. 73, wing; 74, claw; 76, basal lobe; 78, hypopygium.

Aedes campestris Dyar and Knab

Figs. 68, 69, 70, 73, 74, 76, 78

Description

This species is occasionally referred to as the salt and pepper mosquito.

Proboscis with black scales on the distal half and white scales near the base. Mesonotum (Fig. 68) with a broad median bronzy brown stripe and lateral brown patches. Wing scales (Fig. 73) mainly white, with scattered black scales, R_{4+5} white scaled. Legs largely white scaled near the base, dark scaled at the end; tarsal segments with basal and apical white rings. Claw ratio (Fig. 74) 1.3 : 0.7; second tooth long and parallel to main tooth. Abdomen of female (Fig. 70) white with black patches, segment seven entirely white scaled. Abdomen of male (Fig. 69) with white T-shaped markings on segments two to four only, remainder of segments largely white.

Hypopygium (Fig. 78). Apical lobe small, rounded with long setae. Basal lobe somewhat cup-shaped with numerous slender setae (Fig. 76).

Notes on Biology and Distribution

Aedes campestris is common in the dry plains areas of the American northwest. It breeds abundantly in waters that are rich in organic matter and decidedly on the alkaline side (Fig. 158). For that reason the species may be restricted in general distribution, although extremely abundant in a particular area. The larvae are generally associated with those of *A. dorsalis*.

It is a very common species on our prairie in late May and early June. At that time it is surpassed in abundance only by *A. spencerii* Theo. It is the main mosquito on the alkali flats around the Quill Lakes. As Mail (23) has shown, the eggs of this species remain viable over long periods of time. This probably explains its presence in great numbers in flooded hay meadows which have been dry for a period of years. Attention is called below to the extraordinary mosquito situation at the Dundurn military camp in 1943. Saskatchewan records are numerous: Aberdeen, Aneroid, Arran, Borden, Cedoux, Dafoe, Dundurn, Fox Valley, Kenosee, Kindersley, Lang, Maple Creek, Regina, Rosthern, Saskatoon, Scott, Stockholm, White Fox.

The adults are on the wing in early June. They are usually found resting on the grass and will attack fiercely when disturbed. They will bite readily in the hot afternoon, as well as in the evening. They are long-lived and rubbed specimens may be encountered a month after the initial emergence. If conditions are favorable, a second brood appears in late August.

Aedes vexans (Meigen)

Figs. 79, 80, 81, 82

Description

Proboscis brown. Vertex with fine white and brown scales and numerous erect forked yellow scales, cheeks with white appressed scales. Mesonotum entirely bronzy-brown. Wing scales black with some white scales on base of

costa and along inner edge of costa for half its length. Tarsi black with narrow basal white rings. Abdomen of female (Fig. 82) black with two rounded white patches at the base of each segment and a triangular patch at the posterior margin of segments five to seven. Abdomen of male (Fig. 81) black with basal white patches which become long triangles on posterior segments.

Hypopygium (Figs. 79, 80). Dististyle narrow basally, widening toward the apex, claw inserted subapically. Apical and basal lobes absent. Claspette (Fig. 80) capitate, crowned with numerous curved setae.

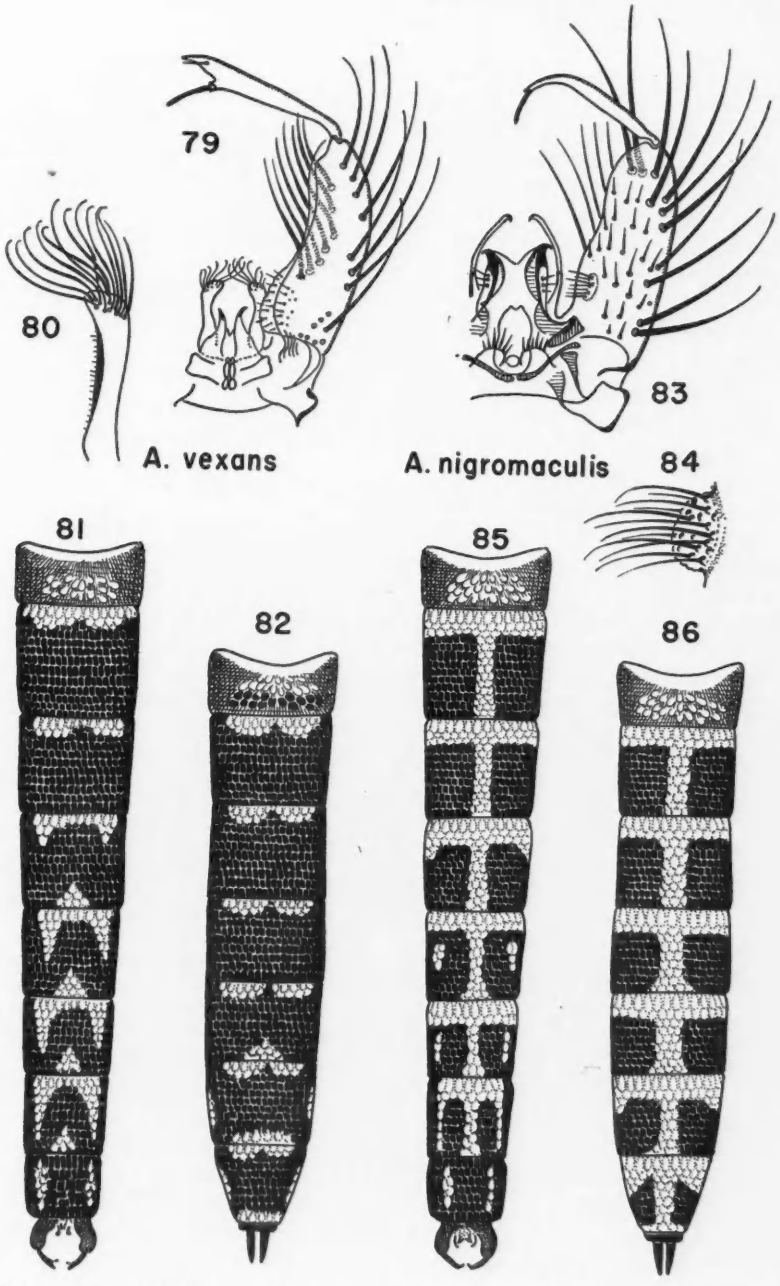
Notes on Biology and Distribution

This is one of the most common species of mosquito in North America north of Mexico. It is also widespread in the Palaearctic and Oriental regions. An extensive summary of its distribution records is given by Natvig (28, pp. 418-420). In Saskatchewan the writer has collected the species in large numbers in the following localities: Dafoe, Estevan, Indian Head, Maple Creek, Melfort, Nipawin, Ogema, Prince Albert, Regina, Saskatoon, Smeaton, Spy Hill, Waskesiu. Although Dyar (3, p. 237) states that this species is not found in the forested Canadian Zone, the writer has encountered the species in large numbers at Waskesiu. In Saskatchewan then the species is found in the three main ecological zones, the prairies, aspen grove region, and coniferous forest. It appears that two forms of this species occur in the province: a small northern one found in the aspen grove and coniferous forest regions which is on the wing from mid June to early July, and a larger prairie form which is common during late July and August. The latter may have a second brood in early September. Although larvae are encountered frequently in shallow pasture depressions of the open prairie following July rains, semi-permanent marshy areas are a favorite habitat.

Aedes vexans is a fierce biter and is one of our most annoying species in midsummer. It migrates readily for long distances. In the lower Columbia River Valley, Stage *et al.* (39) found the species miles from its breeding grounds. In Saskatchewan the two largest cities, Regina and Saskatoon, occasionally suffer an invasion of *A. vexans* in late summer or early fall.

In this species the adaptability to survival is developed to a high degree. Gjullin *et al.* (8) in their excellent study of the biology of this species showed that on the average a female lays more than 40 eggs in a batch and that she may lay more than one batch. They further showed that the eggs may remain viable for a period of several years and that periodic flooding and drying is necessary for a complete hatch. Moreover, it was found that eggs laid during the summer did not undergo a dormancy period, thus making it possible for the species to have several generations in a season.

Because of its wide geographic distribution, its long flight range and its vicious feeding habits, *Aedes vexans* must be considered as one of our most important species.



FIGS. 79-82. *Aedes vexans*. 79, hypopygium; 80, paraproct; 81, abdomen of male; 82, abdomen of female. FIGS. 83-86. *Aedes nigromaculis*. 83, hypopygium; 84, basal lobe; 85, abdomen of male; 86, abdomen of female.

Aedes nigromaculis (Ludlow)

Figs. 83, 84, 85, 86

Description

Proboscis black with a broad white band in the middle. Vertex with fine white hairs near the middle which gradually shade into brown near the sides; numerous erect forked white scales on the nape; cheeks with white appressed scales and a small patch of black scales near the center. Mesonotum with a broad median bronzy-brown stripe, remainder mainly yellowish white. Wing scales predominantly white on costa, subcosta, and radius. Legs black with broad basal white rings on the tarsal segments. Abdomen of female (Fig. 86) black with basal white bands and a broad white median longitudinal bar. Abdomen of male (Fig. 85) similar, but white markings becoming T-shaped on segments four to seven.

Hypopygium (Fig. 83). Apical lobe absent, basal lobe (Fig. 84) a small flattened area bearing setae. Filament of claspette narrow and long.

Notes on Biology and Distribution

A. nigromaculis is common on the great plains areas of North America. In Saskatchewan it is restricted to the southern parts with the northern border of its distribution running through the Regina district. Our records are as follows: Weyburn, Trossachs, Yellow Grass, Regina, Vanguard, Lafleche. It breeds in shallow pasture depressions and weedy irrigation ditches. The first brood is encountered during July, while a second emergence may take place in mid-September, if conditions are favorable. In the Regina area this second brood is occasionally very troublesome.

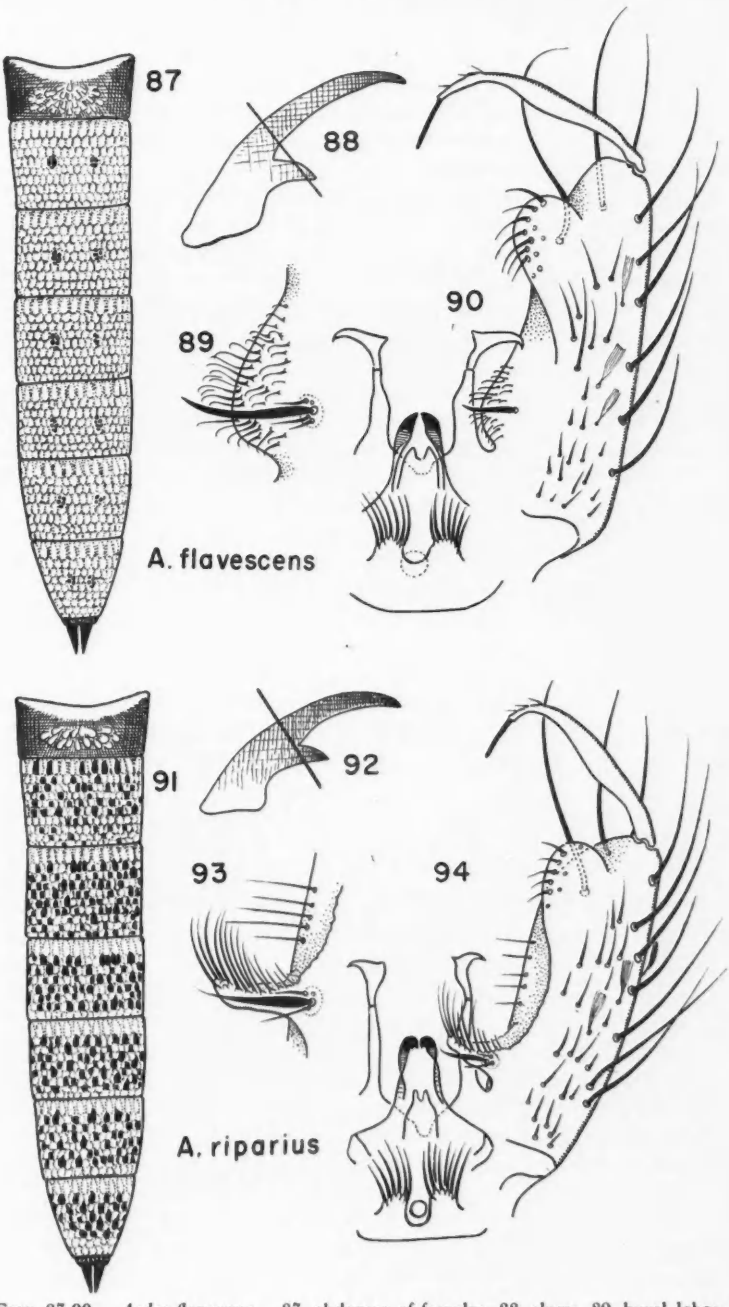
This species is a severe biter, and the writer has found it to attack when its abdomen is still distended with blood from a previous feeding. This behavior has not been observed in the case of any other species.

Aedes flavescens (Müller)

Figs. 87, 88, 89, 90

Description

Proboscis with black and white scales intermingled. Vertex with fine golden scales; nape with forked yellow erect scales; cheeks with closely appressed white scales and a few scattered black scales. Mesonotum with golden brown scales, a median broad band of bronzy brown scales; antescuteller area covered with fine white scales. Wings with white and black scales, anterior veins largely white scaled, R_{4+5}, M_{1+2}, and M_3, cubital fork and $2A$ largely black scaled. Legs with white and black scales on femora and tibiae; tarsal segments black with basal one-third to one-half of each segment white. Claw ratio 1.1 : 0.9 (Fig. 88). Abdomen (Fig. 87) dorsally white scaled with two small black patches on each segment; venter white scaled with scattered black scales forming a broad median longitudinal bar.



FIGS. 87-90. *Aedes flavescens*. 87, abdomen of female; 88, claw; 89, basal lobe; 90, hypopygium. FIGS. 91-94. *Aedes riparius*. 91, abdomen of female; 92, claw; 93, basal lobe; 94, hypopygium.

Hypopygium (Fig. 90). Basistyle stout. Apical lobe prominent, with retrorse setae. Basal lobe (Fig. 89) low, rounded with numerous fine curved setae aud one prominent spine. Claspette short, expanded along basal two-thirds of stem, filament angularly expanded beyond neck.

Notes on Biology and Distribution

A. flavescens has a wide geographic distribution in North America. It is found from Alaska south to California, across Canada and the northern states to Labrador and New York. It also has a wide distribution in Europe and Asia (28, pp. 272-273).

In Saskatchewan this is one of the most common pests of the prairie area. It is especially abundant on the grasslands south of Yellow Grass to Weyburn. Here conditions for breeding and feeding of this species appear to be ideal. The level and poorly drained land has extensive areas covered with sedge and slough grass, a preferred habitat. In the shallow sheltered areas the water warms up rapidly in the spring and a generation emerges before they become dry. These same areas are later used for oviposition. As range lands these flats support large herds of cattle which constitute the preferred host.

Although the adults bite when their habitat is invaded, only rarely are specimens encountered in towns and cities. Before settlement, this species must have been extremely annoying to the buffalo that roamed over the plains.

Larvae are found in mid-May in semipermanent water. The writer has collected the larvae consistently in small sloughs overgrown with sedge (*Carex* sp.), *Scirpus* sp., slough grass (*Beckmannia* sp.), and water hemlock (*Cicuta* sp.) in which the water was stagnant, rich, and alkaline (Fig. 154). Adults appear generally in June, disappear during July and may reappear as a small second brood in August. Hearle (13) has made an extensive study of the bionomics of this species.

Saskatchewan records include: Aberdeen, Arran, Athapapuskow, Borden, Consul, Cudworth, Dafoe, Dundurn, Eastend, Fox Valley, Garrick, Humboldt, Indian Head, Kindersley, Laird, Langham, Love, Maple Creek, Nipawin, Piapot, Regina, Robsart, Rosthern, Saskatoon, Waskesiu, Welby, White Fox, Yellow Grass.

Aedes riparius Dyar and Knab

Figs. 91, 92, 93, 94

Description

Proboscis black, with numerous scattered white scales, especially on basal two-thirds. Vertex with fine white and brown scales; cheeks with white appressed scales and a patch of black near the center; nape with numerous brown forked scales. Mesonotum a coppery brown, sometimes with an indication of a darker brown broad median stripe. Wings with black and white scales, the former predominating. Legs with black and white scales; first tarsal segment with a small patch of white scales at the base on the outside surface; segments two, three, and four, with a broad basal white ring,

remainder black; last segment entirely black. Claw ratio 0.9 : 1.1 (Fig. 92). Abdomen (Fig. 91) with black and white scales intermingled, with slight indication of basal white rings on all segments and apical white rings on segments four to seven.

Hypopygium (Fig. 94). Apical and basal lobes present. Basal lobe (Fig. 93) projecting mesad, posterior surface concave, a row of long curved setae along the margin and one stout spine near the base of the lobe.

Notes on Biology and Distribution

This species occurs from Ontario west to Alberta, north through the Yukon into Alaska, and south to Colorado and Wisconsin. It is also found in Europe. In Saskatchewan the writer has collected larvae and adults in the area from Saskatoon north to Lac la Ronge. The records include: Arran, Lac la Ronge, Langham, Love, Lake Lenore, Quill Lake, Saskatoon, Waskesiu, White Fox. From the above it appears that *A. riparius* is at home in the transition zone between forest and prairie. The larvae were found most commonly in small weedy ditches and depressions in the neighborhood of clumps of aspen poplar. Larvae with exceptionally long gills are not uncommon.

This is an early species and adults are on the wing from mid- to late May. The biology of the species is imperfectly known.

Aedes communis (De Geer)

Figs. 95, 96, 97, 98

Description

Proboscis black. Vertex with fine curved yellow scales; nape with many yellow forked erect scales; cheeks with appressed yellowish-white scales. Mesonotum (Fig. 95) generally with two narrow brown stripes bordered on either side by yellowish white scales; a very fine median black bare line separates the white scales between the mesonotal stripes. Wing scales black. Legs black. Abdomen black with basal white bands that widen laterally on the last few segments.

Hypopygium (Fig. 98). Basistyle long and narrow. Apical lobe with long setae. Basal lobe (Fig. 97) with curved spines along the margin and, dorsally, with a long and prominent curved spine and several small setae; posterior surface of basal lobe concave. In *A. pionips* Dyar (Fig. 101) the posterior surface of the basal lobe is convex and there are several long spines on the dorsal margin, although one is slightly larger than the others. Claspette (Fig. 96) curved, stem gradually narrowing from base to apex (cf. Fig. 100).

Palpi of male longer than proboscis and last segment enlarged (46).

Notes on Biology and Distribution

This species has an exceptionally wide geographic distribution in the nearctic and palearctic regions. In Saskatchewan it has not been encountered on the prairie, it is rare in the aspen parklands, but common in the coniferous

forested areas. It is one of the most frequently encountered species at Waskesiu in early spring. Other northern records are Lac la Ronge and Amisk Lake.

This is an early species. Larvae appear soon after the snow begins to melt and adults are on the wing by the middle of May.

Aedes pionips Dyar

Figs. 99, 100, 101, 102

Description

Proboscis very long and black. Vertex with narrow white and brown scales; cheeks with appressed white or yellowish-white scales; nape with numerous forked yellow erect scales. Mesonotum (Fig. 99) with two broad black or dark brown stripes separated by a fine line of yellowish scales; two black patches at the sides of the antescutellar area; remainder of mesonotum white scaled. Wing scales purplish-brown. Legs with exception of under surface and tip of femora black, with purplish reflection. Abdomen black with basal white rings which distinctly widen laterally on segments four to seven.

Hypopygium (Fig. 102). Basistyle long and narrow. Apical lobe long and narrow with long setae. Basal lobe (Fig. 101) covered with fine spines which are only slightly curved; dorsal margin with several spines, one of which is slightly larger than the others; posterior surface of lobe convex and covered with fine hairs. Claspette (Fig. 100) curved, expanded along basal two-fifth.

Male palpi subequal or shorter than the proboscis (Vockeroth (46)).

This species is closely related to *A. communis* and was for some time considered a variety of it. Recently Vockeroth (46) has made a detailed comparative study of the two species and comes to the conclusion that "the large number of associated differences indicates that *pionips* is not a variety of *communis* but is a distinct species" (p. 246).

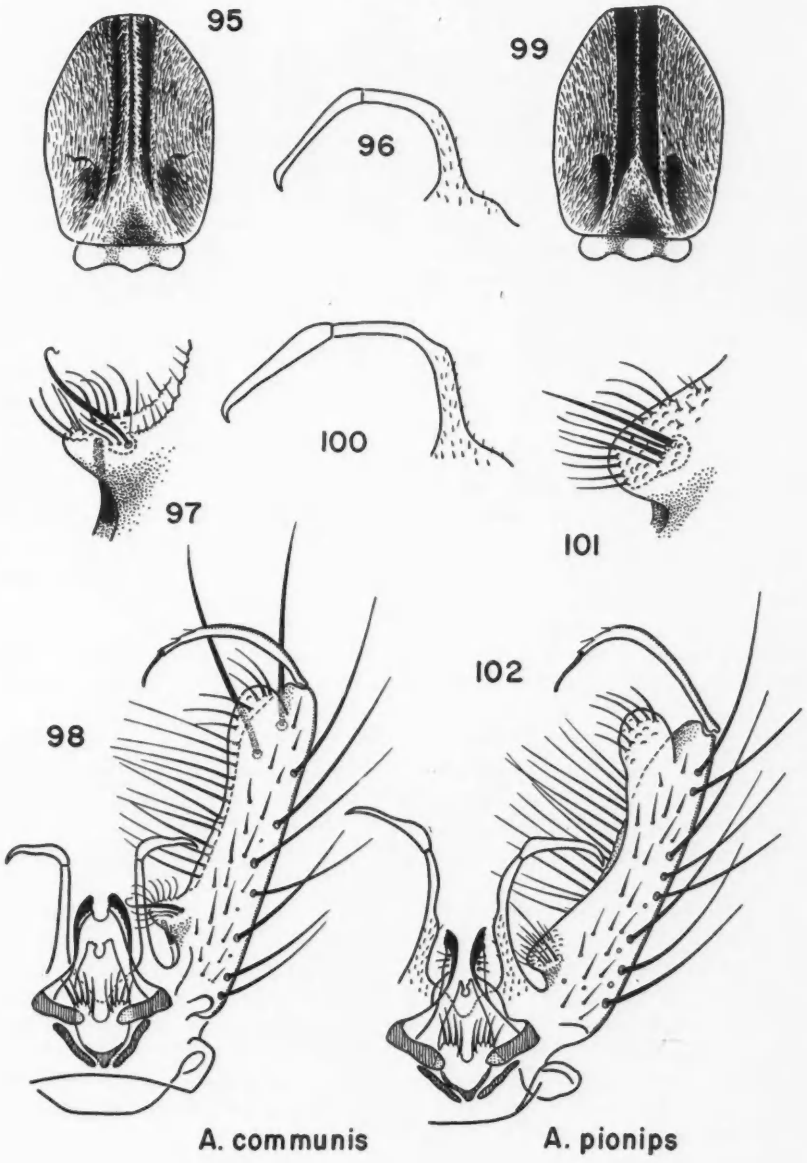
Notes on Biology and Distribution

This species has a geographic distribution which extends from Alaska southeast through the Northwest Territories and forested areas of the western provinces to Hudson Bay and from northern Quebec to Labrador. In the mountain areas of the west the range of the species extends into Colorado.

In Saskatchewan this species is rare in the aspen parkland, but common in the coniferous region from Prince Albert north. Our records include: Regina, Prince Albert, Nipawin, White Fox, Waskesiu, Lac la Ronge, Smeaton, Emma Lake.

The larvae of this species are found in late season. The writer has collected larvae in large numbers as late as the middle of July. A common breeding place is cool and clear water in small depressions at the bases of spruce trees.

Our observations indicate that the species rarely if ever takes blood. On July 10-12, 1943, the writer made an extensive collecting trip in the Prince Albert and neighboring districts. At Melfort, White Fox, and Smeaton,



FIGS. 95-98. *Aedes communis*. 95, mesonotum; 96, claspette; 97, basal lobe; 98, hypopygium. FIGS. 99-102. *Aedes pionips*. 99, mesonotum; 100, claspette; 101, basal lobe, 102, hypopygium.

Aedes pionips adults were frequently observed to settle on the observer. They did not attack exposed portions, but settled on the clothing. They appeared to be nervous and quickly departed if disturbed. This contrasted sharply with the actions of two other species, *Aedes fitchii* and *A. canadensis*, which were most persistent under the same conditions.

A similar condition was observed on July 15 in a spruce forest just north of Prince Albert. It was a cool morning (temperature 50° F.) and showery. Many *A. pionips* adults were found clinging to the needles of the lowest branches of spruce trees. Many of the females had their abdomens distended with a grayish material, presumed to be of vegetative origin. This was considered probable because a few females had parts of small flowers fastened to the proboscis.

Aedes cataphylla Dyar

Figs. 103, 104, 105, 106, 109*a*, 110*b*

Description

Proboscis dark brown. Vertex with numerous fine brown scales and a few white ones; cheeks with yellowish white broad appressed scales and a patch of black scales near the center. Mesonotum (Fig. 104) with two faintly indicated dark brown stripes, sides shading from golden brown to white. Wings with dark scales, a small patch of white scales on the base of the costa. Legs black, femora mainly white on underside and at apex, tibiae and first tarsal segments sprinkled with white scales, remainder black. Abdomen (Fig. 103) black with basal white rings that broaden laterally on the posterior segments; venter white with triangular black patches at the posterior corners of each segment.

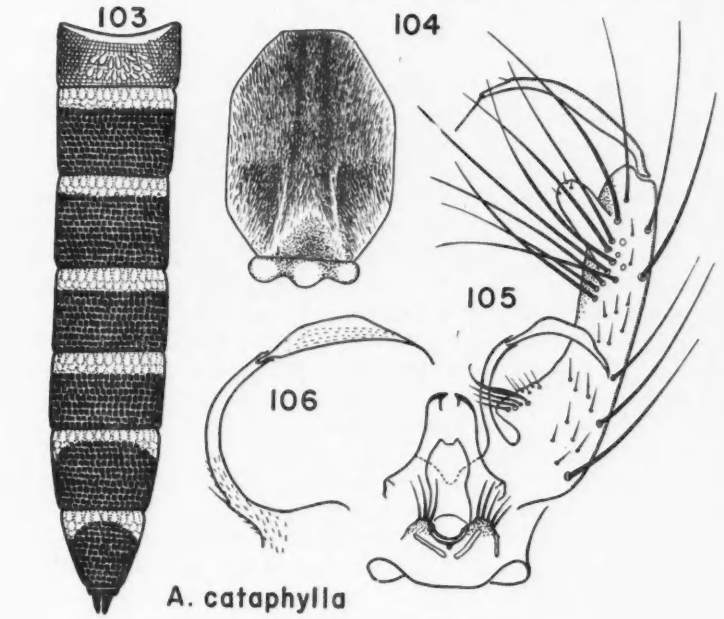
Hypopygium (Fig. 105). Basistyle, narrow with a tuft of long hairs at the base of the apical lobe. Apical lobe long and narrow, almost bare. Basal lobe cone-shaped with one spine and long setae (Fig. 110*b*). Claspette (Fig. 106) with highly curved stem and long filament which is slightly expanded near the middle and has the outer edge parallel to the inner edge.

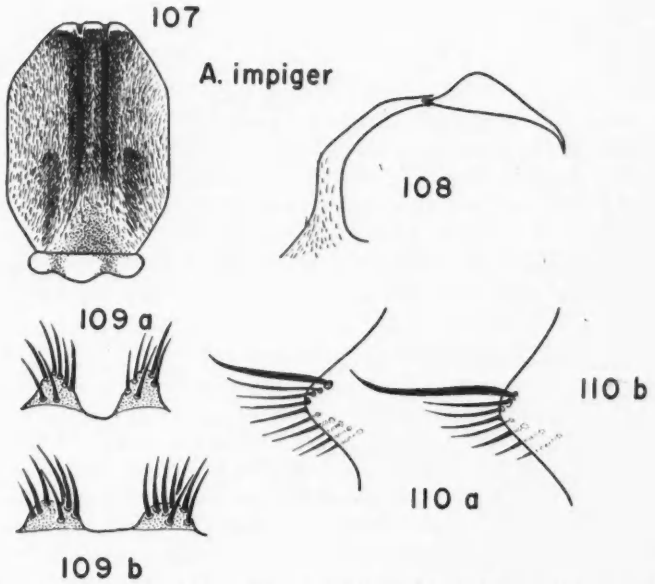
Notes on Biology and Distribution

In North America this species has a distribution extending from the western border of the Great Plains west across the mountains, north into Alaska, and south into California and Utah. In its southern range it is restricted to high elevations. It has a wide distribution in Asia (Siberia) and Europe (Natvig (28, p. 293)).

In Saskatchewan it is found on the prairie in the southwest of the province and in the aspen parklands of central Saskatchewan extending north into the coniferous forest zone. The records are: Eastend, Val Marie, and Cabri from the southwest; Dundurn and Lake Lenore from the parklands; Waskesiu from the forested zone.

The larvae have been found in small grassy depressions sheltered by bluffs. They appear in late April and adults are on the wing in early May. It is a





FIGS. 103-106, 109*a*, 110*b*. *Aedes cataphylla*. 103, abdomen; 104, mesonotum; 105, hypopygium; 106, claspette; 109*a*, lobes of ninth tergite; 110*b*, basal lobe. FIGS. 107, 108, 109*b*, 110*a*. *Aedes impiger*. 107, mesonotum; 108, claspette; 109*b*, lobes of ninth tergite; 110*a*, basal lobe.

rare species in the province. The late Eric Hearle, in a personal communication to Dr. K. M. King, dated Oct. 24, 1929, states that *A. cataphylla* is the main troublesome species in the Banff (Alberta) district, that it will move considerable distances in search of a blood meal and does not confine its activities to the shelter of woods. He further states that it is troublesome by day or night and is a very facultative breeder.

Aedes impiger (Walker)

Figs. 107, 108, 109*b*, 110*a*

Description

Proboscis dark brown. Vertex with few narrow white scales, a conspicuous black patch of scales on either side of the median line, cheeks black, edged with white. Mesonotum (Fig. 107) with white scales, with a broad median light brown band and two dark stripes, the latter begin anteriorly as two impressed bare lines; in most specimens there is a conspicuous group of white scales on the anterior margin of the mesonotum. Wing scales black. Legs black, with scattered white scales on femora, tibiae, and first tarsal segments. Abdomen black, with conspicuous basal white bands.

Hypopygium similar to that of *A. cataphylla*. Basistyle long and narrow with a tuft of long hairs arising near the base of the apical lobe. Apical lobe narrow, long, almost bare. Basal lobe (Fig. 110*a*) cone-shaped, with a row of setae, the first one slightly larger than the others and spinelike. In *A. cataphylla* the spine is considerably longer than the setae. Claspette (Fig. 108). Stem bent elbow-like; filament conspicuously enlarged near base and tapering gradually toward apex. According to Matheson the filament has a prominent toothlike projection near the base. The writer has no male specimens in his collection. The description is based on specimens collected at Fort Nelson, B.C., by W. R. Mason, on loan from the Canadian National Collection, Ottawa. These specimens lack the toothlike projection. Moreover the writer has a hypopygial mount of a specimen bearing the label Ottawa River, Seig. Club, Que., May 3, 1936, and identified by Dr. Matheson as *A. impiger* (Wlk.). This specimen also lacks the toothlike projection. It would appear then that the above character is not uniformly present. The lobes of the ninth tergite (Fig. 109*b*) are low, each with seven to nine spines. In *A. cataphylla* the lobes (Fig. 109*a*) are more narrow and pointed and each has from three to seven spines.

Notes on Biology and Distribution

A. impiger is a forest loving species. It is found in forested regions of Canada and the northern United States. The records include: Quebec (41); New York (26); Minnesota (29); Montana (24); Manitoba (Dyar, quoted by McLintock) (22); Colorado (26); Yukon (3). It is not a common species. The Saskatchewan records of adults are as follows: Beaver Creek, 8-VI-1928, A. E. Cameron; Waskesiu, 26-V-1938, J. G. Rempel. Observations on the larval habitat have been made by Owen (29), by Jenkins and Knight (19), and by Mail (24). It is an early species.

Aedes trichurus (Dyar)

Figs. 111, 112, 113, 114

Description

Proboscis black. Vertex with numerous fine white scales. Mesonotum (Fig. 111) with brown and white scales intermingling, giving it a "frosted" appearance. Wings black scaled. Legs purplish black, except basal two-thirds of femora which have many white scales. Abdomen black with basal white rings which widen slightly laterally.

Hypopygium (Fig. 114). Basistyle markedly curved. Apical lobe a small outgrowth of the tip of the basistyle. Basal lobe (Fig. 113) double, a low cone bluntly rounded, with apical setae, and a pointed cone with setae arising at different levels of the cone. Claspette (Fig. 112) long and sinuate, slightly swollen before apex, with transverse ridges at the end.

Notes on Biology and Distribution

This species has a distribution limited to the wooded regions of southern Canada and the northern United States (26). In Saskatchewan all records are from Waskesiu. The writer has a series of adults collected May 26, 1938. In 1929 Dr. K. M. King collected larvae on May 16 and adults as late as July 2. This suggests that the species is an early one, but that the adults are fairly long-lived. The larvae have been collected in small weedy sloughs associated with *A. punctor*, *A. excrucians*, and *A. fitchii*.

Aedes intrudens Dyar

Figs. 115, 116, 117

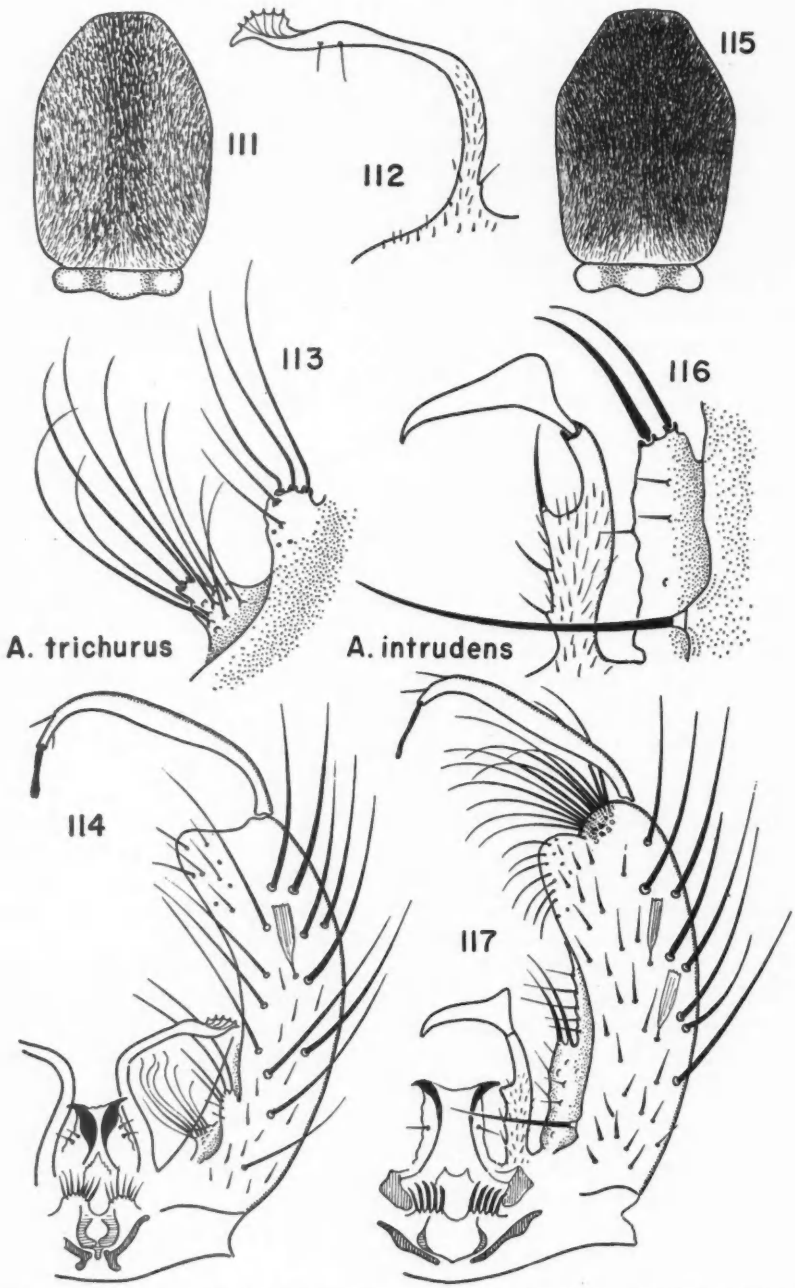
Description

Proboscis dark brown. Vertex with fine white scales; cheeks with broad white appressed scales. Mesonotum (Fig. 115) uniformly grayish to bronzy brown. Wings dark scaled. Legs with exception of inner surface and tips of femora purplish brown. Abdomen with broad basal white bands which widen slightly laterally; brown scales with purplish reflection.

Hypopygium (Fig. 117). Basistyle apically with a patch of long hairs. Apical lobe with long setae. Basal lobe (Fig. 116) directed caudad, ending in two prominent spines, and one large spine near its base, directed mesad. Claspette (Fig. 116) broadly expanded along basal two-thirds, angulate, with a short seta; filament foot-shaped.

Notes on Biology and Distribution

This species is found in the forested areas of Canada from the Atlantic (Labrador) west to the Rocky Mountains. Its distribution extends north into Alaska and south into the northern states (Michigan, Wisconsin, Minnesota) and along the mountain ranges into Utah and Colorado. In Saskatchewan it is common in the spruce forests from Emma Lake north to



FIGS. 111-114. *Aedes trichurus*. 111, mesonotum; 112, claspette; 113, basal lobe; 114, hypopygium. FIGS. 115-117. *Aedes intrudens*. 115, mesonotum; 116, basal lobe and claspette; 117, hypopygium.

Lac la Ronge. In the extensive collection of adults made at Waskesiu during the last two weeks of July, 1930, by K. M. King and the author, *A. intrudens* formed almost fifty per cent of the population. In the same year the species was on the wing from June 5 to Aug. 18. It is an annoying species by day and night and will travel some distance in search of food.

Aedes canadensis (Theobald)

Figs. 118, 119, 120

Description

Proboscis dark brown. Vertex with fine white scales along median line, shading into brown at the sides; cheeks with flat yellow appressed scales. Mesonotum (Fig. 119) bronzy brown with white scales at the sides and around the antescutellar area; in some specimens there is an indication of two yellowish lines on the disk. Wings with black scales. Legs black with conspicuous narrow white basal and apical tarsal bands. Abdomen (Fig. 118) black with purplish reflection and with triangular white spots on the anterior angles of the segments.

Hypopygium (Fig. 120). Basistyle stout. Apical lobe large and low, evenly rounded, with setae. Basal lobe large, quadrate, with numerous bladelike setae. Claspette short, stem narrow apically, filament narrow and pointed.

Notes on Biology and Distribution

This is one of the most widespread mosquitoes of North America. Its distribution blankets the larger part of the United States and southern Canada. It is a woodland-loving species. In Saskatchewan it is found from Regina, where it is rare, through the aspen grove areas into the lower limits of the coniferous forest zone. It is most abundant near the northern edge of the aspen grove lands.

The seasonal distribution of *A. canadensis* extends from late May until the end of July. The adults attack readily and are most annoying in dense poplar bluffs.

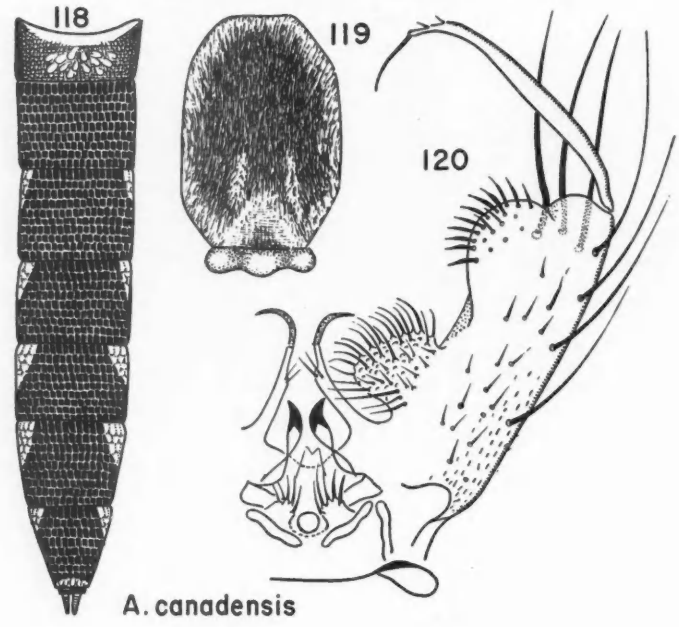
The Saskatchewan records are: Emma Lake, Melfort, Nipawin, Pleasantdale, Prince Albert, Regina, Saskatoon, Smeaton, Waskesiu, White Fox.

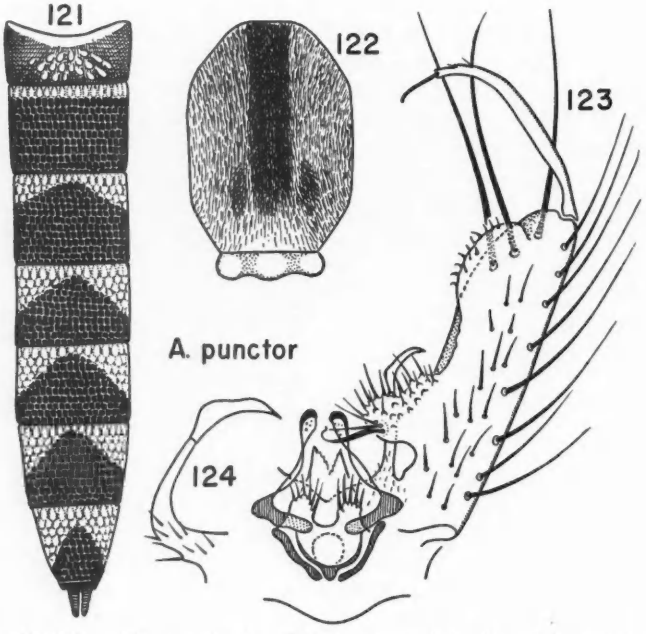
Aedes punctor (Kirby)

Figs. 121, 122, 123, 124

Description

Proboscis dark brown. Scales on vertex, cheeks, and nape almost entirely yellowish white. Mesonotum (Fig. 122) in fresh specimens with yellowish scales and a broad median bronzy-brown stripe, the latter may be faintly divided longitudinally by a narrow yellow line; in specimens stored for some time the median stripe is golden brown and the sides are white. Wings with dark brown scales. Legs black, apices of femora white. Abdomen (Fig. 121)





FIGS. 118-120. *Aedes canadensis*. 118, abdomen of female; 119, mesonotum; 120, hypopygium. FIGS. 121-124. *Aedes punctor*. 121, abdomen of female; 122, mesonotum; 123, hypopygium; 124, claspette.

black with basal white bands which widen laterally into triangles; venter white scaled, each segment with an apical black band which widens in the middle into a black triangle.

Hypopygium (Fig. 123). Basistyle quite stout. Apical lobe large with very short curved hairs. Basal lobe large, apically expanded, densely covered with setae and with one curved spine on the dorsal margin. Claspette (Fig. 124) curved, filament only slightly expanded.

*Notes on Biology and Distribution**

A. punctor is found in the northern forested areas of the United States, Alaska, Canada, Asia, and Europe. Natvig (28, pp. 340-344) discusses the distribution records of this species in great detail. In Saskatchewan this species has been collected at Waskesiu and at Lac la Ronge. In May, 1952, Mr. D. R. Oliver of the Fisheries Research Field Laboratory at Lac la Ronge made extensive larval collections for the writer. Most of the larvae were *A. punctor*, indicating that this is one of the dominant species of the area. Jenkins (15) finds that in central Alaska this is the most abundant species.

A. punctor is said to be a fierce biter. According to Natvig (28, p. 346) this species "is probably the most persistent biter of the Fennoscandian mosquitoes and in many localities this species is very annoying. In the lowlands this mosquito mostly attacks in the evening and in the night or, in shaded places by day, but in swampy areas in the high mountains, I have been attacked by hundreds, even on hot days, in bright sunshine. In mountainous regions *A. punctor* is very often found in houses, cow-stables and hog-pens and cause great annoyance both to man and beasts, especially at night."

In Canada extensive observations on the bionomics of this species have been made by Hocking *et al.* (14), by Haufe (11), and by Jenkins and Knight (19).

Aedes triseriatus (Say)

Figs. 125, 126, 127

Description

Proboscis black or dark brown. Vertex with fine white scales; nape with forked yellowish white scales. Mesonotum (Fig. 126) silvery white with a very broad median brown stripe that merges posteriorly with lateral stripes, fine silvery scales cover the remainder of the disk and border the antescutellar area. Wing scales black. Legs black, apices of femora and basal two-thirds along inner surface of femora white. Abdomen (Fig. 125) black, segments four to seven laterally with rounded patches of white.

Hypopygium (Fig. 127). Apical lobe absent, replaced by a group of long setae. Basal lobe absent, its place taken by a row of long setae. Claspette with narrow curved stem and narrow, pointed filament.

** Dr. Kenneth L. Knight has recently published his detailed study of the Aedes punctor subgroup of North America in the Ann. Entomol. Soc. Am. 44 : 87-99. 1951. He finds that Aedes punctor (Kirby) has two varieties which he calls the "type punctor" and "tundra" varieties. The material from Saskatchewan agrees with the description given for the former.*

Notes on Biology and Distribution

An extensive study of this tree-hole breeding mosquito was made by Jenkins and Carpenter (17). According to these authors this species is the most abundant of the tree-hole breeders in North America and is found throughout the deciduous forests and across the great plains in the north. It is stated that the species on occasion is a serious biter and that the bite is painful. The larvae are found in the water of tree holes of a long series of deciduous trees. They have also been found in the pitcher plant and artificial containers (26).

The map given by Jenkins and Carpenter would indicate that this province is outside the known limits of its distribution. However on Aug. 7, 1943, the writer collected a female specimen at "Hungry Hollow", a wooded valley eight miles northwest of Regina. This locality was visited only once and it is possible that the species is fairly common here earlier in the season. The habitat would appear to be favorable for the breeding of this species.

Aedes sticticus (Meigen)

Figs. 128, 129, 130, 131

Description

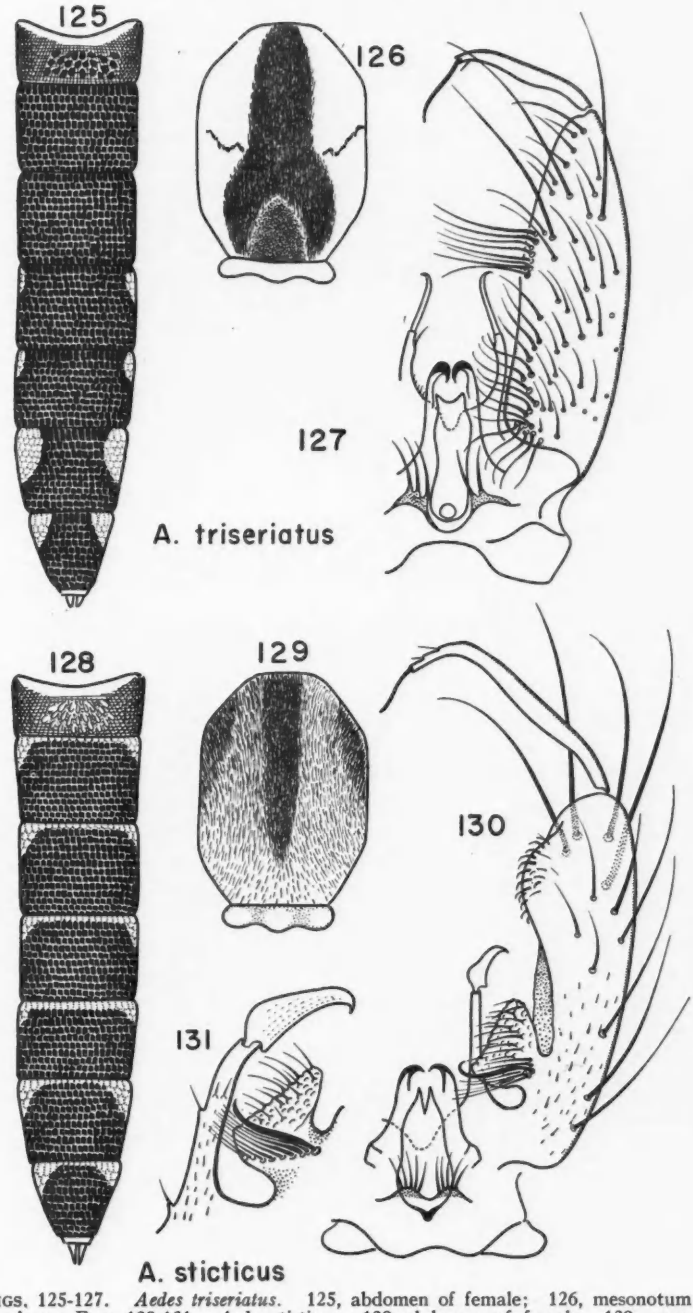
Proboscis dark brown. Vertex with fine white scales; cheeks with grayish broad appressed scales. Mesonotum (Fig. 129) white with a broad median brown stripe which sometimes is divided by a fine pale line into two stripes; white scales on disk shade laterally through yellow into brown, especially near the front. Wing scales brown. Legs brown, front and middle legs entirely white scaled along posterior surface of femora, tibiae, and first tarsal segments; posterior surface of hind legs with dark preapical band on femora, and white scales intermingled with black scales on tibiae and first tarsal segment. The white scaling along the posterior surface of the legs seems to be characteristic of the species. Abdomen (Fig. 128) black, with narrow basal white bands that widen laterally.

Hypopygium (Fig. 130). Dististyle long, narrow, and rather sharply bent near apex. Apical lobe low with short retrorse setae. Basal lobe (Fig. 131) deeply constricted near base, with numerous short curved setae on top and a row of long prominent setae along the anterior margin, preceded by a stout curved spine. Claspette (Fig. 131). Stem fairly straight, of even width, with two setae on the mesad surface; filament basally expanded dorsally and ventrally.

Notes on Biology and Distribution

The synonymy of this species has been the subject of much dispute. It is discussed at length by Natvig (28, pp. 347-349) and by Gjullin (7, p. 230). It is now generally agreed that *A. hirsuteron* (Theobald) and *A. aldrichi* Dyar and Knab are synonyms of *A. sticticus* (Meig.). Moreover, *A. lateralis* (Meig.) has also been synonymized with *A. sticticus* (Meig.) (7).

The species has a wide distribution in the United States, in southern Canada, Siberia, and northern Europe. The larvae are frequently abundant in flood



FIGS. 125-127. *Aedes triseriatus*. 125, abdomen of female; 126, mesonotum; 127, hypopygium. FIGS. 128-131. *Aedes sticticus*. 128, abdomen of female; 129, mesonotum; 130, hypopygium; 131, basal lobe and claspette.

pools of river valleys, hence the name of floodwater mosquito. A fine study of the bionomics of this species in the Lower Columbia River Valley has been made by Gjullin *et al.* (8). According to Ross (36) the females are fierce biters.

In Saskatchewan the species is common in a wooded valley subject to flooding near Trossachs. Isolated specimens have been collected by the writer at Regina and at Ketapwa. In the spring of 1952 Mr. G. Burgess found larvae at Govenlock, Maple Creek, and Cabri in late May. The writer has two female specimens collected at Waskesiu on June 13 and June 18, 1938, and identified by Dr. Matheson as *A. aldrichi* D. and K. These specimens are badly rubbed making positive identification impossible.

Aedes nearcticus Dyar

Figs. 132, 133, 134, 135

Description

Proboscis dark brown. Vertex with fine white and brown scales; cheeks with broad appressed yellow scales. Mesonotum (Fig. 133) with bronzy brown scales, intermingled with white or yellow scales with scattered white scales on outer surface of femora and tibiae, inner side of femora largely white scaled. Abdomen (Fig. 132) black with broad basal white bands which widen laterally on segments six and seven; venter white scaled, with posterior margin of segments black.

Hypopygium (Fig. 134). Basistyle narrow, with long hairs along inner margin. Apical lobe bare. Basal lobe small, posteriorly concave, with setae along margin and a single curved spine. Claspette (Fig. 135) narrow, curved, filament broadly expanded near base, gradually tapering toward apex.

Notes on Biology and Distribution

This species is found in the arctic tundra and in alpine meadows of mountains. Its distribution extends from Alaska south through the Yukon into mountain areas of Alberta, Montana, Washington, Oregon, and Utah, and east across the treeless areas of the Northwest Territories to Ellesmere Island, Greenland, and Labrador. The writer collected a series of adults at Waskesiu in the spring of 1938. This material is in Dr. Matheson's collection at Ithaca, N.Y. The writer has on loan seven male and two female specimens collected by the late Eric Hearle at Banff, in late May, 1922. The species is found in Europe and Asia (28).

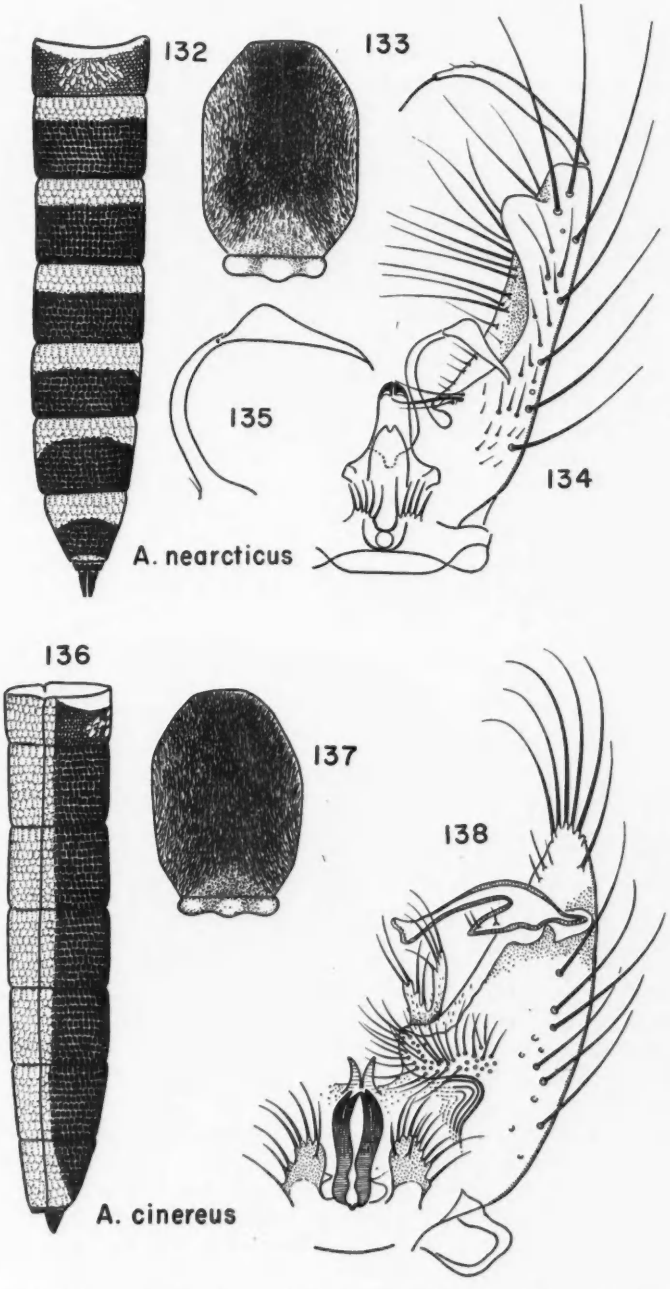
The larvae may be found in small shallow pools filled with water from melting snow. The adult is a fierce biter and may bite at all hours of the day (18).

Aedes cinereus Meigen

Figs. 136, 137, 138

Description

Proboscis dark brown. Vertex with a patch of dark brown scales on either side of the median line, bordered by white scales; cheeks with broad appressed



FIGS. 132-135. *Aedes nearcticus*. 132, abdomen of female; 133, mesonotum; 134, hypopygium; 135, claspette. FIGS. 136-138. *Aedes cinereus*. 136, abdomen of female, lateral aspect; 137, mesonotum; 138, hypopygium.

yellow scales; nape with many brown forked scales. Mesonotum (Fig. 137) uniformly brown. Wing scales brown. Legs dark brown, with white scales on posterior surface of femora and tibiae. Abdomen (Fig. 136). Tergites dorsally black, laterally with white patches that form a continuous white line; venter white scaled.

Hypopygium (Fig. 138). Basistyle broad proximally, somewhat cone-shaped, apex with long setae. Dististyle bifurcate, inserted subapically. Basal lobe large, with many long setae. Claspette greatly modified, bifurcate apically, each branch with a few prominent apical and subapical setae.

Notes on Biology and Distribution

This species has an exceptionally wide geographic distribution in North America. It has been found in Alaska and Labrador in the north and as far south as Arkansas and Florida. It has been reported from most northern states and from most provinces of Canada. It is also widely distributed in Europe and Siberia (28). In Saskatchewan it is a common mosquito at the northern edge of the aspen grove region, but it has not been found on the open prairie. It is a late species, with larvae appearing in early July and adults being on the wing after the middle of July.

Our records include: Dafoe, Lac la Ronge, Melfort, Nipawin, Pleasantdale, Prince Albert, Smeaton, Waskesiu.

Aedes diantaeus H.D. and K.

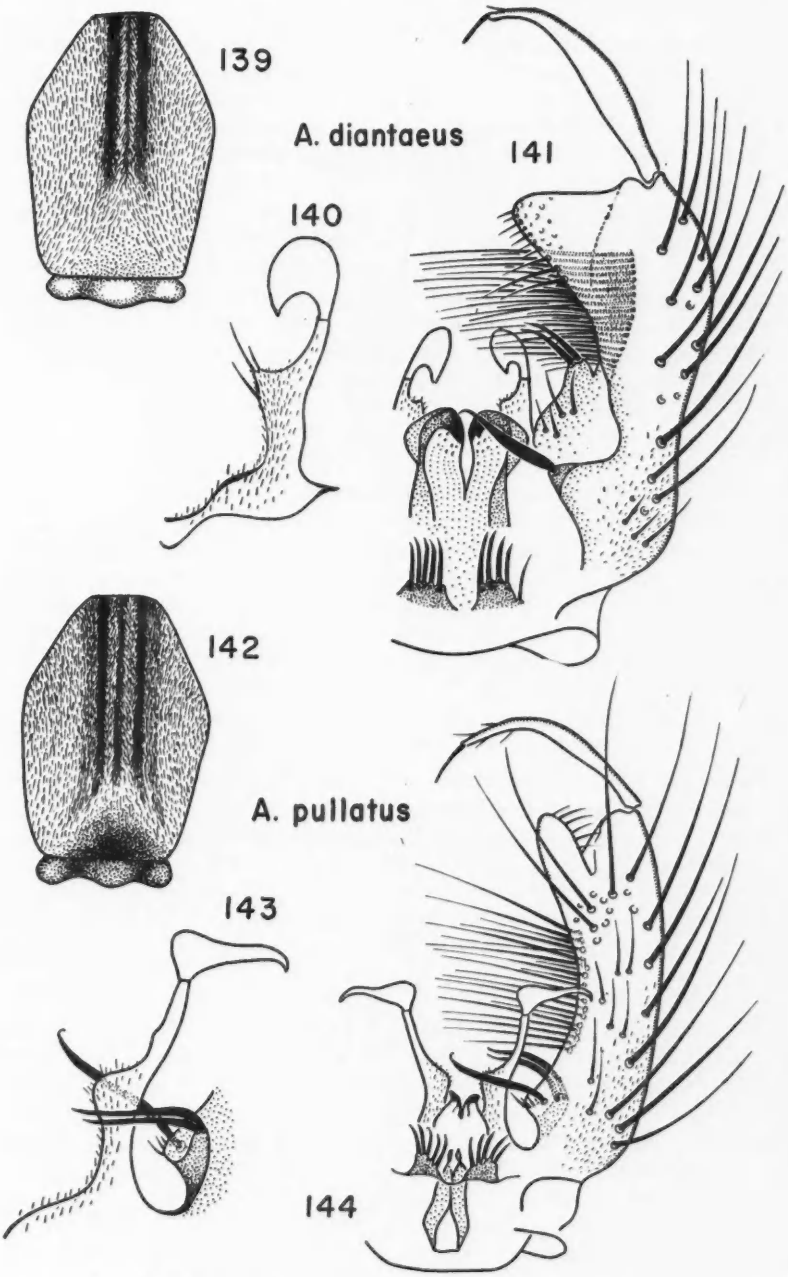
Figs. 139, 140, 141

This species has been redescribed by Smith (38). The Saskatchewan material belongs to this species, and not to the closely related *A. pseudo-diantaeus* Smith.

Description

Proboscis brown scaled. Vertex with numerous fine white scales, nape with white erect forked scales; cheeks with appressed white scales. Mesonotum (Fig. 139) covered with fine yellowish white scales; paired mesonotal stripes brown, narrow, and divided by two narrow yellowish white bands. Wing scales dark. Legs dark purplish-brown with exception of coxae, trochanters, and ventral surface of femora, which are white scaled. Abdominal segments with dark purple-brown scales; tergites laterally with basal white triangular patches; sternites white scaled; each with an apical median triangular black patch.

Hypopygium (Fig. 141). Apical lobe prominent, cone-shaped, with long setae along mesal margin. Basal lobe with two stout spines at the apex and one large spine at the base. Ventral surface of basistyle with a prominent brush of setae. Claspette (Fig. 140). Stem of claspette setose, expanded beyond middle and narrow at apex; filament a flat, very broad, and rounded blade.



FIGS. 139-141. *Aedes diantaeus*. 139, mesonotum; 140, claspette; 141, hypopygium.
FIGS. 142-144 *Aedes pullatus*. 142, mesonotum; 143, basal lobe and claspette; 144, hypopygium.

Notes on Biology and Distribution

According to Smith (38) this species has the following distribution: forested areas of northern United States from Maine to Montana and Wyoming; Canada, Labrador, and Alaska; northern Europe and the Russian Far East. Records from Western Canada include Manitoba, British Columbia, and Northwest Territories. The Saskatchewan material was collected by Dr. K. M. King at Waskesiu, May, 1929, and identified by the late Eric Hearle. The writer has had an opportunity to re-examine the larval material and finds it to agree with the redescription given by Smith (38).

This is an early species. The Saskatchewan material is labelled as follows: "Waskesiu, Sask., May 16-17, 1929. Small slough fed by springs in which much moss was present." One adult was collected at Waskesiu Narrows on May 18, 1929. Considering the extensive collecting at Waskesiu over a period of years, the species appears to be rare in the area.

Aedes pullatus (Coquillett)

Figs. 142, 143, 144

Description

Proboscis black. Vertex with numerous white and brown scales; nape with forked brown scales. Mesonotum (Fig. 142) with a very narrow median black bare stripe and two lateral narrow dark stripes which are frequently bare; mesonotal scales on either side of the bands bronzy brown, laterally shading into yellowish scales. Wings black scaled, with a few white scales on base of costa. Legs largely black; femora white except dorsally and at apex. Abdomen black with narrow basal white bands which widen laterally on segments four to seven.

Hypopygium (Fig. 144). Apical lobe long, bare dorsally, with prominent setae on ventral surface. Basistyle with a row of long hairs on the inner lateroventral surface. Basal lobe with a long curved spine and several small setae on the dorsal surface, and two spines on its ventral prolongation. Fig. 143 is a ventral view of the basal lobe. Claspette sharply elbowed near the middle, setose basally; filament foot-shaped. Lobes of the ninth tergite prominent, heavily sclerotized, with spines along the margin.

Notes on Biology and Distribution

This species has generally been considered a western species, with a distribution extending from Alaska south to Montana. However, Jenkins and Knight (18) found it in abundance on the eastern shore of Hudson Bay (Great Whale River). In the southern limits of its distribution it is restricted to high elevations. Jenkins and Knight (18) have made extensive observations on the biology of the species.

The Saskatchewan records are based on female specimens collected by the writer on June 13, 1944, and identified by him as *A. pullatus*. In the extensive collection of adults made at Waskesiu by Dr. K. M. King on July 2, 1929,

there is one female specimen identified by the late Eric Hearle as *A. pullatus*. Since neither larvae nor males have been found by the writer, the species is recorded here with some hesitation.

Aedes spencerii (Theobald)

Figs. 145-152

Description

Proboscis dark brown. Vertex with fine yellowish scales; cheeks with broad appressed yellowish scales; nape with numerous yellow forked scales. Mesonotum (Fig. 145) with broad median bronzy brown stripe; remainder of disk whitish. Wings (Fig. 146) with black scales on *C*, *R*, R_{4+5}, apices of M_{1+2} and M_3 and *Cu*; scales on remainder of veins white. Legs largely pale scaled. Claw ratio 1 : 1 (Fig. 148). Abdomen of female variable in color, tergites may be entirely white scaled, or with black patches on anterior ones (Fig. 150). Abdomen of male (Fig. 149) largely black with rows of white scales near anterior and posterior margins of segments four to eight.

Hypopygium (Fig. 151). Apical lobe broadly rounded, with short retrorse setae. Basal lobe (Fig. 152) narrowed at base, surface of lobe with numerous curved setae, a large curved spine on dorsal margin. Claspette (Fig. 147). Stem straight, gradually narrowing toward apex; filament sickle-shaped.

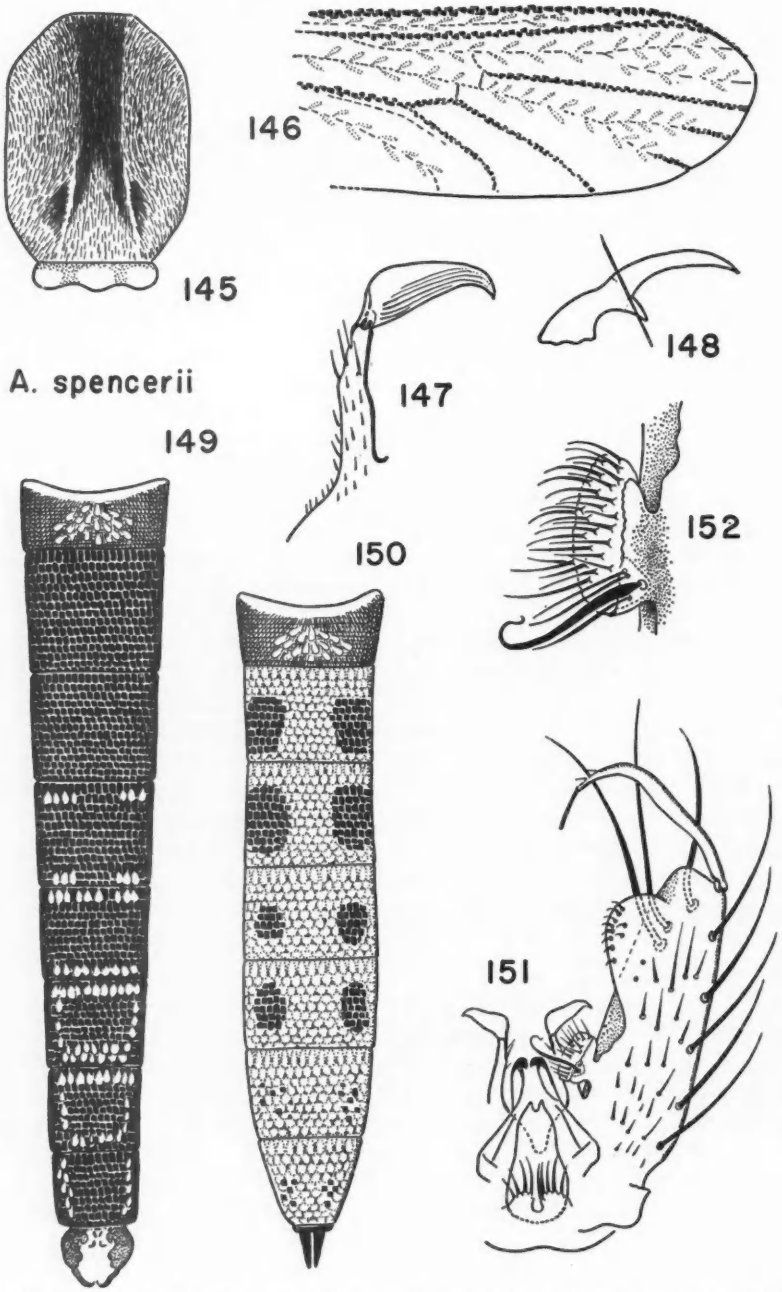
Notes on Biology and Distribution

A. spencerii is the great pest of the prairies of the American northwest. In Saskatchewan it is the dominant species on the prairie in the spring and early summer. It extends in steadily decreasing numbers north thorough the aspen grove area into the coniferous forests as far as Waskesiu.

On the Saskatchewan prairies this is the earliest, most abundant, and widespread species. It breeds predominantly in shallow grassy depressions and overgrown roadside ditches (Fig. 153). Larvae are common during the latter part of April and by May 10 adults are generally present in sufficient numbers to constitute a problem. In the Regina area a second brood has been observed in July and, if there is sufficient rainfall, a third brood may appear in early September.

Although one of our smallest species, *A. spencerii* is the most annoying. It is a fierce biter and feeds readily on hot sunny afternoons. It was found to invade the city of Regina more readily than any other species, causing much inconvenience to people in gardens and on lawns. Our observations indicate that it will travel several miles in search of food. Similar observations were made at Dundurn by Drs. K. M. King and A. P. Arnason of the Dominion Entomological Laboratory at Saskatoon. They found *A. spencerii* to be extremely troublesome in the sand hills at a point nearly three miles airline in all directions from the nearest breeding place.

The records for Saskatchewan are too numerous to list.



FIGS. 145-152. *Aedes spencerii*. 145, mesonotum; 146, wing; 147, claspette; 148, claw; 149, abdomen of male; 150, abdomen of female; 151, hypopygium; 152, basal lobe.

Distribution of Species

Topography, Climate, and Vegetation of Saskatchewan

This account is based largely on the Soil Survey Report No. 12 prepared by Prof. J. Mitchell and co-workers and published by the University of Saskatchewan (27).

The province of Saskatchewan extends from 49° to 60° North latitude and roughly from 101° to 110° West longitude. It is the central region of the so-called prairie provinces of Canada. The southern part of the province is part of the Great Plains region and is characterized by level and gently undulating topography. The northern part of the province and occupying roughly two-fifths of the total area, lies on the Precambrian Shield. It is a region of lakes and rivers, forest and rocks. In the southwest corner of the province (Fig. 160) are the Cypress Hills with an elevation up to 4000 ft. above sea level. From here the land slopes in a northeasterly direction. At the Missouri coteau, an escarpment that runs roughly from the southeastern corner of the province in a northwesterly direction, the elevation is 2000 ft. East of the coteau lies the second prairie steppe characterized by level topography. In it are found the finest agricultural lands of the province.

Conforming to the slope of the region, the drainage systems are directed east toward Hudson Bay. The two main river systems are the Saskatchewan with its north and south branches, and the Churchill. The southeastern corner is drained by the Qu'Appelle River which flows east into Manitoba and ultimately drains into Lake Winnipeg. This small stream flows in a large valley at one time occupied by the Saskatchewan River. Only the southwest, the Cypress Hill region, has a drainage flowing south. In the interior of the province some drainage is effected through small streams that drain into alkaline lakes. Because of extremely level topography, many extensive areas have poor drainage. These areas may produce tremendous populations of mosquitoes in the spring and early summer.

Location and topography to a large extent determine the climate of a region. Saskatchewan, being far from the ocean and being cut off from the moisture-laden air currents of the Pacific by the Rocky Mountains, has a continental climate. This climate is characterized by extremes in temperature. The summers are hot and dry, the winters are cold, with little snow. The temperature difference between summer maximum and winter minimum may be as great as one hundred and fifty degrees on the Fahrenheit scale. Not only is the total annual precipitation low, but much of it is lost by hot dry winds in the summer. Although the average annual precipitation may vary markedly with different localities in the province, 15 inches may be taken as a representative figure. Almost fifty per cent of this falls during June, July, and August.

On the basis of its native vegetation the province may be divided into four main regions, the lines of division running roughly from southeast to northwest (Fig. 162). The southernmost area is occupied by the prairie characterized by a vegetation cover of low grasses and by an absence of trees. The soil is

brown (Fig. 161). The second region, the park belt, is characterized by a grassland cover interspersed with aspen groves. The soil is black. About two-thirds of the province is in the northern coniferous forest region where the dominant vegetation consists of jack pine, spruce, and poplar on a gray soil. The northeastern corner of the province lies within the Northern Transition Forest zone.

Geographic Distribution

In Saskatchewan the most intensive collecting of mosquitoes has been concentrated in the Regina and Waskesiu areas. Lac la Ronge has been visited on several occasions. Over a period of years periodic collecting trips have been made through the aspen grove zone between Saskatoon and Prince Albert. During the spring of 1952 the writer made extensive larval collections in the Saskatoon and surrounding areas. In the same season, at the request of the writer, a number of University students made extensive larval collections in other parts of the province. From these data Table I was drawn up showing the distribution of our species in the three main ecological zones. The writer is confident that this represents a reasonably complete picture of the situation. It should be pointed out, however, that the area investigated extends only as far north as Lac la Ronge.

Table I indicates that the dominant species of our mosquitoes belong to the genus *Aedes*. With the exception of *Culex tarsalis* and *Culiseta inornata* on the prairie, representatives of other genera are quite rare. In each zone several species dominate the picture. Thus on the prairie they are *A. spencerii*, *A. campestris*, and *A. flavescens*, closely followed by *A. vexans* and *A. dorsalis*. In the aspen grove region the place of *A. spencerii* is taken by *A. fitchii*. In northern wooded areas, the so-called "black-legged" species predominate.

The mosquito fauna of a particular region depends upon a complex of factors. This complex will include environmental conditions such as temperature, rainfall, wind, and more specific conditions such as soil type, pH of water, richness in organic matter, presence or absence of vegetation. It can therefore be seen that even on the open prairie considerable local variations in the mosquito fauna may still exist. Of our prairie species only *A. spencerii* is uniform in distribution. *A. flavescens* is found concentrated in regions with semipermanent sloughs, while *A. campestris* breeds in large numbers in alkaline depressions rich in organic matter. For this reason on the prairie a species rare in one region may be a pest in another. In our northern regions, however, where habitats overlap to a greater extent, in any region the mosquito population is likely to consist of a number of species.

Seasonal Distribution

The sequence of appearance of adult mosquitoes varies in the three main ecological zones of the province.

On the prairie there may be a marked succession of species, and a succession of broods of individual species, with the first appearing in early May and the last emerging in early September. The sequence fluctuates markedly from

TABLE I

GEOGRAPHIC DISTRIBUTION OF SASKATCHEWAN MOSQUITOES

Ecological zone	Species		
	Abundant	Common	Rare
Prairie grasslands. Soil brown, zones 1 and 2. Total summer ppt. 10-12 in.	*A. spencerii* *A. campestris* *A. flavescens*	*A. dorsalis* *A. vexans* *A. nigromaculis* *Culex tarsalis* *Culiseta inornata*	*A. fitchii* *A. increpitus* *A. excrucians* *A. canadensis* *A. cataphylla* *A. sticticus* *A. triseriatus* *Culex restuans* *Culiseta morsitans* *Psorophora signipennis* *Anopheles earlei*
Aspen grove region. Soil black, zones 3 and part 4. Total summer ppt. 12-13 in.	*A. fitchii* *A. campestris* *A. flavescens*	*A. spencerii* *A. vexans* *A. canadensis* *A. cinereus* *A. excrucians* *A. dorsalis*	*A. pionips* *A. communis* *A. riparius* *A. increpitus* *A. impiger* *A. stimulans* *A. cataphylla* *Culex tarsalis* *Culex restuans* *Culiseta inornata* *Anopheles earlei*
Northern coniferous forests. Soil gray zones parts 4 and 5. Total summer ppt. 12-14 in.	*A. intrudens* *A. communis* *A. punctor* *A. pionips*	*A. fitchii* *A. canadensis* *A. excrucians* *A. cinereus* *A. cataphylla* *A. vexans* *A. trichurus*	*A. spencerii* *A. cinereus* *A. riparius* *A. flavescens* *A. cataphylla* *A. impiger* *A. diantaeus* *A. pullatus* *A. nearcticus* *Culiseta* spp. *Culex territans* *Mansonia perturbans* *Anopheles earlei*

year to year being greatly influenced by the amount and distribution of rainfall. The succession outlined here is that found in a year with normal rainfall. The first species to appear is *A. spencerii* with adults generally on the wing by mid-May. It may have a second brood in mid-July and a third in late August or early September. Whether the second and third broods come from eggs laid in the previous year or from eggs laid by the first brood is not known. *A. campestris* emerges during late May. *A. flavescens* appears in late May or early June. *Culiseta inornata* is found in June in small numbers. Generations may follow in quick succession, resulting in a large population by fall. Although *Culex tarsalis* appears a little later, it is generally concurrent with *C. inornata*, since their breeding habits are similar. *A. dorsalis*,

A. vexans, and *A. nigromaculis* appear in July with a possible second generation in late August or early September. In the prairie area the numbers of mosquitoes are thus subject to extensive and irregular fluctuations.

At the southern edge of the aspen grove zone the first great emergence of mosquitoes takes place in mid-May. This population is made up largely of the following species: *A. spencerii*, *A. fitchii*, *A. campestris*, and *A. flavescens*. *A. riparius* and *A. excrucians*, although present, are never found in large numbers. The above species persist for the remainder of May and throughout June. *A. vexans* becomes common in July. If heavy rains occur in early September, the sudden outbreak is caused by a second brood of *A. vexans*. At the northern edge of the aspen grove zone the first great emergence, in mid-May, consists of *A. fitchii*, *A. communis*, *A. campestris*, *A. spencerii*, and *A. flavescens*. A few weeks later this is followed by *A. canadensis*, *A. cinereus*, and *A. pionips*. *A. vexans* is again one of the last to appear.

In the northern wooded areas most species emerge in late May or early June and persist till the end of July. Each species has only one generation. The first half of the summer, June and July, is therefore noted for its great mosquito abundance, while the second half, August and September, is more or less mosquito free.

Adaptation to Survival

Hibernation

The species of *Aedes*. These form the dominant group in every region of the province at any time of the mosquito season. In all our *Aedes* species the egg is the overwintering stage. The eggs are laid singly on the ground where there is rank vegetation. Relative humidity must play a part in the selection of the place of oviposition. On the grassy prairie the females select the low lying places. Since the soil moisture content is higher here than in the surrounding areas, the relative humidity amongst the vegetation is also higher. The final result is that the eggs are deposited in areas where sooner or later there will be water. The number of eggs laid by one female varies greatly, and the figures stated in the literature are derived from the numbers laid by individuals in captivity or from the numbers counted in the ovaries of gravid females. Gjullin *et al.* (8) found that the average number of eggs laid by 214 *A. vexans* under laboratory conditions was 43 and that some females laid several batches. Hocking *et al.* (14) counted as many as 170 eggs in dissected specimens of *A. punctor* and as many as 300 in *A. excrucians*.

In the great majority of our species there is only one generation a season. Hence the eggs laid in the summer normally do not hatch until the following spring. In these species the embryo goes into diapause which is broken only through desiccation. If snow and rainfall are insufficient to fill the low lying areas with water next spring, the eggs lie dormant for another season without an appreciable loss of viability. In some of our aedine species, such as *A. dorsalis* and *A. vexans*, the embryo does not need to go into diapause. Hence the eggs may hatch in the season in which they are laid and several generations may be produced during one summer.

The species of *Psorophora*. This genus has only one species represented in our mosquito fauna, namely *P. signipennis*. Little is known of the life history of the representatives of this group, although Ross (36) states that all species hibernate in the egg stage and that the larvae of some species are predaceous, feeding on other mosquito larvae.

The species of *Mansonia*. Our single representative of this genus passes the winter in the larval stage under ice. The eggs are laid in rafts on the water.

The species of *Wyeomyia*. Our possible representative species passes the winter in the larval stage frozen in ice in the pitcher plant, *Sarracenia purpurea* L. According to Matheson (26) the females lay the eggs in young leaves in which there is no water.

The species of *Culiseta*. It is generally believed that the members of this group pass the winter as adult females. The adults come out of hibernation in early spring and lay their eggs in rafts on water. In our northern species there is probably only one generation a season, while in *C. inornata*, which is common on the prairie, there may be a number of generations. Since Wilkins and Breland (48) recently reared this species from eggs that had been dry for a considerable time, the possibility that this species may be able to hibernate in northern areas in the egg stage should be considered. It is possible that *C. morsitans* passes the winter in the larval stage (19, 28).

The species of *Culex*. Like the members of the preceding genus, the species of *Culex* overwinter as adult females in a sheltered place. The adults appear in the spring to lay their eggs in rafts on the water. In *C. territans* Walker the hibernating females come out in early June and there seems to be only one generation. The other two species, *C. restuans* Theo. and *C. tarsalis* Coquillett, appear late, generally toward the end of June or early July. They have several generations.

Viability of Eggs

It is well established that in the Western plains areas mosquito abundance fluctuates greatly from year to year. In season of abundant rainfall the mosquito population may reach great proportions. In dry season the populations are not only greatly reduced but, as in the drought year of 1937, mosquitoes may be almost absent. Moreover, in a country with an extreme continental climate, a number of dry years may follow in succession. This was experienced in the dry 1930's in Saskatchewan when from 1931 to 1937 there was a series of dry years. In the season of 1941 and 1942 the rainfall was heavy and mosquitoes were unusually abundant. The question is often asked how mosquitoes are able to survive under those conditions.

The aedine mosquitoes pass the winter in the egg stage. The eggs laid in low-lying weedy depressions in a previous season become flooded in the spring when the snow melts. Not all eggs hatch at the first flooding (Hearle (12, p. 30)), an excellent safeguard should the amount of water be insufficient for complete development of larvae and pupae. Repeated flooding may thus bring out succeeding broods. The eggs hatch most readily in water with low oxygen tension (Gjullin *et al.* (8)). Such conditions generally obtain in water

that is shallow, warm, and rich in organic matter, i.e. water especially suited for larval development. If snow and rainfall are insufficient in one season, the eggs may remain dormant until the following season. Gjullin and his co-workers (8), using *A. sticticus* and *A. vexans*, conducted experiments in the Lower Columbia River Valley which indicated "that eggs of both species will survive in large numbers for two or three years and that small numbers of eggs may survive for four years". This ability to withstand freezing and thawing, wetting and drying over a long period of time, explains the appearance of hordes of mosquitoes in a wet year after prolonged drought. Indirect evidence in this respect was found by the writer in 1943 at the Dundurn military camp. This camp is situated some twenty-five miles south of the city of Saskatoon. The country north of the camp is characterized by rolling sandhills with intermittent poplar bluffs. Immediately south of the area are extensive marshes and five miles due west are the wide stretching river flats with their countless shallow weedy depressions. The area affords excellent breeding habitats for every species of the western plains.

Of particular importance is an extensive marshy area immediately south of the camp. In wet years this is flooded to an extent of about 700 acres and is then called Wapisiu Lake (Fig. 155). In the spring of 1943 this presented a unique problem. According to reports by laymen in the district, and military officials of the camp, the area had been dry for at least seven years and large quantities of hay were harvested each season. No doubt even in dry years, areas of moist ground were widespread. Such grassy spots would be excellent places for mosquito oviposition. Several factors might contribute to egg deposition on a large scale. The district normally produces serious mosquito outbreaks caused by emergence from the numerous shallow depressions in the poplar bluffs. Livestock in the pastures south of the camp and army personnel on the march provide countless mosquitoes with blood. Moreover, oviposition takes place in the most convenient and attractive place. The open meadow, "Wapisiu Lake", with its heavy covering of grass and moist ground, would be such a place.

In the spring of 1943, partly as a result of melting of heavy snows, and partly as a result of the heavy rainfall in the previous summer, this area was flooded. Some seven hundred acres of meadow land were covered to an average depth of four feet. The mosquito eggs laid here over a period of years, hatched. On May 11 the lake was found to be infested at a rate of

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FIGS. 153-158. Typical breeding places of prairie mosquitoes. Photos courtesy Dominion Department of Agriculture. 153. A shallow slough, the water is stagnant, alkaline, and rich in organic matter. The vegetation consists of sedges and slough grass. A breeding place of *A. spencerii, A. campestris, A. dorsalis, A. vexans, Culiseta inornata*, and *Culex tarsalis*. 154. A small slough overgrown with sedge, slough grass, and water hemlock. *A. flavescens* breeds here. 155. Edge of Wapisiu Lake, 700 acres of flooded hay meadow. *A. campestris* is found here in tremendous numbers. 156. Numerous small depressions overgrown with wild barley and alkali grass at the outskirts of the city of Regina. The water is extremely rich in organic matter (total solids 3812 p.p.m.). Mainly *A. dorsalis* and *A. campestris*. 157. Boggy Creek near Regina. *A. increpitus* breeds in the stagnant depressions at the edge of the stream. 158. A long shallow slough in pasture land. The edges are heavily encrusted with alkali. *A. campestris*.

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three hundred larvae per cubic foot at all depths and all over the lake. Most of the larvae, later identified as *A. campestris*, with a few *A. dorsalis*, were in the second or third instar. They were in a weakened condition judging by the difficulty experienced in attempting to rear them. This may have been due to the low temperature of the water, 47° F. A great larval mortality took place within the next two weeks. When the area was visited by Dr. K. M. King of the Federal Entomological Laboratory on May 28, the following situation was found. There were fewer than five larvae per cubic foot; these larvae were still in the second or third instar; there were no pupae; no adults had emerged. Whether this great reduction followed the application of over 2000 gallons of oil on May 13-15, or occurred as a consequence of the low temperature of the water, was not ascertained. Although the larvae of the northern black-legged species are most resistant to low water temperatures, *A. campestris* is usually found in shallow pools where the water warms up rapidly during the day. Hence the temperature of the water in this case may have been a contributing factor in the mortality of the larvae. Had the entire mosquito population emerged there would have been some ten million mosquitoes for every soldier in the camp.

Dispersal and Migration

It is well known that certain mosquitoes will travel considerable distances from their breeding places. This tendency is especially well developed in salt-marsh and floodwater species. It is, however, not uncommon for other aedine mosquitoes to fly a distance of several miles.

The earlier observations on mosquito flight range were indirect in nature. A study of the distribution of the breeding habitats of a species, followed later by observations on the distribution of the adults of this species, gave indication of its migration. Rees (32) made numerous observations of this kind in his study of flight range of Utah mosquitoes. On several occasions the writer found *Aedes spencerii* and *A. vexans* to be present in great numbers as larvae a few miles outside the limits of Regina. A few days later the city was invaded by hordes of these mosquitoes. Similar observations were made by the writer at the summer resort of Waskesiu where the mosquito problem has always been serious. Before the introduction of DDT and the "fogging" machine, control consisted in oiling the numerous shallow pools in the vicinity of the camp site and the golf course. This gave considerable relief in the early season. However, a moderate offshore wind blowing for a number of days carried hordes of mosquitoes into the area and quickly nullified earlier control efforts. The species involved were the northern "blacklegged" ones.

In more recent studies of mosquito migration, dyes were sprayed or dusted onto newly emerged adults. The recovery later of such marked mosquitoes at various distances gave direct information on their movements. With the development of the radioactive tracer technique an excellent method for the study of insect migration and flight range has been found. This technique is now widely used.

In the studies of flight range of mosquitoes some writers have given attention to the possible underlying causes, such as light, temperature, relative humidity, and wind. However, there has been no general agreement in the interpretation of the results. According to Provost (30) the disagreement may be due to a failure on the part of investigators to distinguish between *dispersion* and *migration*. He calls the former an appentential flight, i.e. one receiving internal direction, while the latter is a nonappentential flight, a response to an external influence. A physiological state such as hunger may cause a species to fly in search of food apart from, or even in spite of, an external influence. Since at least a limited movement in all directions from the larval habitat has been so consistently observed, we must assume dispersion to be a normal method of distribution for mosquitoes. The second type of movement, namely migration, has been less frequently observed. It is most pronounced in the so-called salt-marsh mosquitoes of coastal areas. However Rees (32) has shown that in other aedine mosquitoes such as *A. dorsalis* and *A. vexans* migrations of varying magnitude may occur. The writer's frequent observations of mosquito flight at Waskesiu suggest that the northern black-legged species also migrate.

Feeding Habits of Mosquitoes

The interest in mosquitoes is based mainly on the fact that they are blood-sucking insects. Through their persistent attack, mosquitoes, especially when present in large numbers, become extremely annoying to man and beast. Vivid accounts have been written by early explorers about mosquitoes of the Canadian arctic. In our northern coniferous areas mosquitoes are a great pest every year during June and early July. Although the plough and harrow have upset many of the breeding places on the plains, during the early days of the settlement of our west, mosquitoes were a scourge to the pioneer.

The realization that mosquitoes may transmit disease, in addition to the annoyance caused by their attack, has led to extensive studies of their feeding habits. Various techniques have been employed in these studies. Field observations disclosed that some species feed on plant nectar in addition to blood. Thus Hearle (12, p. 39) noticed that in the case of *A. aldrichii* D. and K. (= *A. sticticus* Meig.) "both sexes feed on nectar and plant juices and are especially attracted to the flowers of white spiraea". In the studies of the woodland species in the spring of 1943 we found that *A. pionips* did not attempt to take blood and that a fairly large number had floral parts attached to the head, indicating that they had visited flowers. In recent years this field has been investigated extensively by Canadian workers at Churchill. In their studies of the bionomics of northern mosquito species, Hocking *et al.* (14) found that many female mosquitoes carried pollinia of the woodland orchid *Habenaria obtusata* Pursh. Their studies suggested that "no fewer than 74% of the mosquitoes attracted to man at the middle of July, 1948 had fed on *Habenaria obtusata*" (p. 77). West and Jenkins (47) have shown that males and females of *Aedes communis* under laboratory conditions take up P^{32} from

the flowers of plants, the stems of which have previously been immersed in a solution of this radioisotope. However, proof that such mosquitoes can lay viable eggs is still to come.

A study of feeding habits has thrown light on the possible role mosquitoes play in the transmission of certain diseases. The precipitin test is a widely used tool in these studies. Using this technique, Hammon and Reeves (10) have shown that *Culex tarsalis* feeds extensively on avian hosts thereby strengthening the hypothesis that this species is an important vector of the virus of Western equine encephalomyelitis from bird to mammal. Rempel *et al.* (35) showed that the following prairie aedine species feed extensively on several avian and mammalian hosts: *A. spencerii*, *A. flavescens*, *A. vexans*, *A. nigromaculis*, and *A. dorsalis*.

It is thus evident that mosquitoes are not only most efficient in securing food, but that this food may come from a variety of animal and plant hosts.

Factors Influencing Mosquito Abundance

Many factors may contribute toward the presence or absence and toward the rise or fall of the mosquito population in an area. Since the larvae are aquatic, it follows that precipitation will be an important factor. However, a correlation between total rainfall and mosquito abundance does not follow. The amount of standing water is the difference between "gain and loss". If the land is dry, the water will be soaked up so quickly that little will be left in depressions. Thus on June 27, 1945, a 3½-in. "cloudburst" at Regina caused very little standing water in the extensive prairie depressions immediately north of the city because of the dryness of the two preceding seasons. On the other hand, the extensive breeding places in 1943 in various parts of the province were largely the result of the high soil moisture content following a heavy snow-fall in the preceding winter and excessive rainfall during the previous summer. Again, if the precipitation falls in the form of a slow, prolonged drizzle, little water will accumulate. If a rain is followed by strong warm winds, evaporation will quickly dispose of the water in the shallow places bringing all breeding to an end. Moreover, it has been found repeatedly that maximum mosquito development takes places when the habitat has been subjected to alternate drying and flooding. It is well established that not all eggs hatch at the first flooding. Only alternate drying and flooding assures the hatching of the remainder. Hence continued submergence will result in a decreasing hatch. This factor operates also in another way. It has been noticed by the writer in the laboratory and in the field, that an abundance of filamentous algal growth results in marked larval mortality. Again algae are most abundant in the permanent bodies of water. Therefore, alternate drying and flooding, resulting in the destruction of algae, will, in turn, bring about favorable conditions for mosquito development.

In the seasons of 1940 and 1943 the precipitation for the summer months (April–September, inclusive) was below average. Moreover, this fell mostly in the form of small showers. The water then soaked into the ground or was lost through evaporation before the species could complete their cycle. In

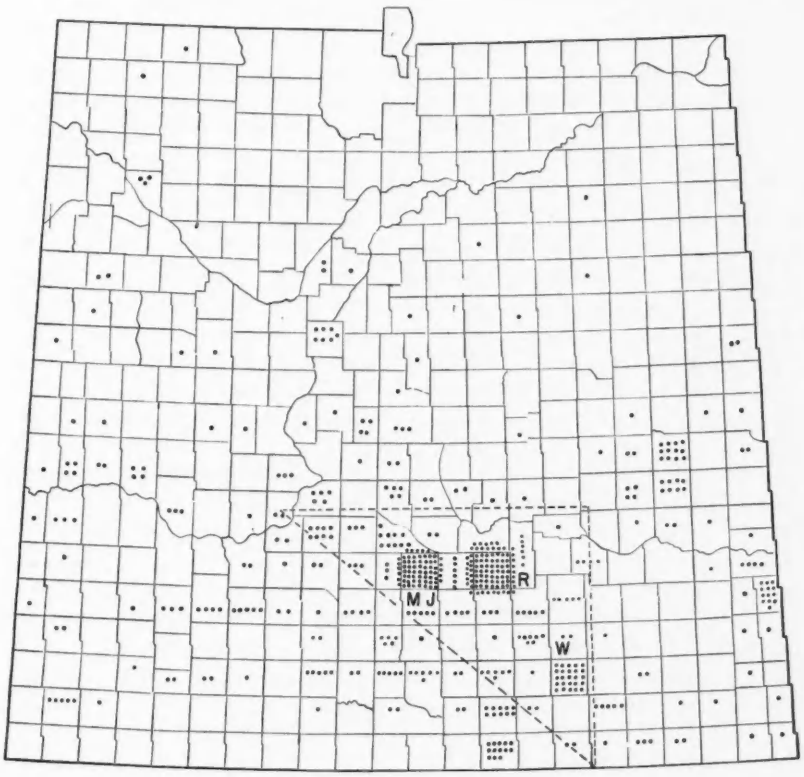
1941 and 1942, the total summer rainfall was above normal, and a great deal of it fell in the form of heavy rains. In 1942 these rains occurred at more or less weekly intervals. This close succession coupled with low temperatures created habitats that retained water for the entire season. As a result, only a fraction of the eggs of each species hatched. Moreover, the adult populations that emerged had their numbers reduced by the frequent heavy rains accompanied by strong winds. In 1941 the heavy rains were three to four weeks apart. This gave most species ample time to complete a cycle.

The second factor that has a great effect on mosquito populations is temperature. Its adverse effect by speeding up evaporation has already been mentioned. Low temperatures may reduce populations by slowing down larval development and adult activity. In addition, very low temperatures may destroy an entire brood. Thus on May 18, 1943, *Aedes spencerii* became extremely troublesome in the Regina area as a result of a great emergence during a period of warm weather. On May 23 very cold weather set in with temperatures near freezing accompanied by rain and strong northerly winds. The pest came to an abrupt end. Similarly, on Sept. 10, 1942, *A. dorsalis*, *A. vexans*, and *A. nigromaculis* became a great plague in the Regina area. Frost two days later destroyed most of them.

Mosquitoes and Western Equine Encephalomyelitis

Western equine encephalomyelitis was first recognized in Saskatchewan in 1935 when it appeared in the form of an epizoötic in horses. According to Fulton (5) the disease may have made its appearance in the province prior to 1935, but cases may have been diagnosed as forage-poisoning, cerebrospinal meningitis, blind staggers, botulism, etc. There were isolated cases of the disease in late summer and fall of 1936. A second epizoötic appeared in 1937 and a third in 1938. According to Fulton the first two outbreaks began in the southeastern corner of the province and spread in a northwesterly direction, suggesting that the disease was introduced from the midwestern states. However in 1938 cases appeared spontaneously in widely separated areas, indicating endemicity for the disease. The latter outbreak was especially severe and it has been estimated that there were some 50,000 equine cases with a loss of 15,000 animals. Vaccination was then undertaken on a large scale and the incidence of the disease dropped to an astonishing degree.

During 1939 and 1940 the province escaped both epidemic and epizoötic outbreaks, although isolated cases no doubt occurred. In the summer of 1941 more than five hundred cases in man were diagnosed (Table II). Since diagnosis of a mild form is difficult, it is possible that the total far exceeded the above figure. The number of fatal cases was 44. Comparatively few equine cases were recorded, probably because of the effective process of vaccination in the years preceding, or because of active immunity acquired through previous infection. During the 10-year period from 1942 to 1952 there have been no outbreaks in the equine and human population comparable



DISTRIBUTION OF HUMAN CASES OF ENCEPHALOMYELITIS IN SASKATCHEWAN, 1941 EPIDEMIC

FIG. 159.

to those of 1938 and 1941. However, there have been local outbreaks in each season "which, in some instances, have been quite severe" (Fulton (6, p. 212)).

Encephalomyelitis is typically a disease of the summer months. In this respect data from Saskatchewan, Manitoba, and the United States are in full agreement. The seasonal aspect of the disease, both in the epizoötic as well as in the epidemic form, has stood out in every case. Thus in the 1935 outbreak (epizoötic) the first cases appeared early in August. "The epidemic increased in intensity until a peak was reached about the middle of September. From that time fewer cases were reported, and the spread of the disease was much less rapid. Toward the end of September several frosts occurred, and almost immediately the outbreak was over" (Fulton (5, p. 2)). There were few cases in 1936 and these appeared in late summer and fall. In the 1937

TABLE II

SEASONAL INCIDENCE OF HUMAN ENCEPHALOMYELITIS IN SASKATCHEWAN, 1941

Date	Number of cases	Date	Number of cases
Month of:		Week of:	
May	1	Sept. 7-13	26
June	1	Sept. 14-20	7
July	4	Sept. 21-27	4
		September, not stated	1
Week of:		Sept. 28-Oct. 4	8
Aug. 1- 2	3	Oct. 5-11	1
Aug. 3- 9	15	Oct. 12-18	2
Aug. 10-16	107	Oct. 27-Nov. 1	1
Aug. 17-23	180	Nov. 9-15	1
Aug. 24-30	109		
August, not stated	33	Total	543
Aug. 31-Sept. 6	39		

outbreak the peak was reached the first week in September. The general epidemiological picture of the 1938 epizoötic resembled that of the previous outbreaks.

The outbreak of the disease in epidemic form closely follows the trend outlined above. In 1935 there were isolated human cases in the summer, mainly from agricultural areas. Although few cases were diagnosed in 1938, the greatest number of these occurred in August. The seasonal aspect of the disease, however, stood out most prominently in the 1941 outbreak (Table II). In Saskatchewan, out of 543 cases, 396 or 72.9% occurred between Aug. 16 and Aug. 30. The seasonal incidence of the disease suggested the probability of mosquito transmission. This was early recognized in the history of the disease in California and led to the outstanding investigations of Hammon and his co-workers. As a result of these extensive studies it is now generally agreed that the disease is mosquito borne and that one species, *Culex tarsalis*, plays a major role. Although, in general, the writer's finding in Saskatchewan are quite in agreement with the theory of mosquito transmission, it is difficult on this basis to account for the widespread epizoötic in the province in the drought year of 1937.

Since extensive observations of the mosquito fauna of the Saskatchewan prairie were begun by the writer only in 1942, our information regarding mosquito abundance prior to this date is very limited. However, some information is available in the records kept by the members of the Dominion Entomology Laboratory at Saskatoon. The writer has consulted the records in the fortnightly reports of the laboratory. Although drought conditions prevailed in various parts of the province during the summer of 1935 (5), there was sufficient rainfall for extensive mosquito breeding. Thus the entry for July 1-16 reads:

"Heavy rains fell in nearly all parts of the province during the first part of the period".

and the entry for July 16-31 is as follows:

> "Many sections of the prairies, which have had no standing water in previous years, now have numerous shallow pools. As a result, mosquitoes are relatively abundant and troublesome. Some farmers state that numbers of mosquitoes approximate those of pioneering days."

The total rainfall for the season of 1935 at Saskatoon was 17.77 in. and at Regina 17.75. Both figures are well above the long-term average. The precipitation for the season of 1938 was normal, while that of 1941 was considerably above normal. Moreover, during the latter two seasons the rainfall came at more or less regular intervals of three weeks, a condition that normally leads to a great mosquito build-up. Against this must be pointed out that the 1937 epizoötic occurred at a time when severe drought conditions prevailed over the larger part of the province. Further, mosquitoes were unusually abundant during parts of 1942 when the disease was absent.

Although a positive correlation between mosquito abundance and encephalomyelitis has not been established, our findings indicate that there is a correlation between weather and disease. Our epidemics and epizoötics have appeared only in hot seasons. An examination of the mosquito theory of transmission in the light of this seems warranted.

Assuming that mosquitoes play a major role in the transmission of the disease, the mean summer temperature might affect the incidence of it in several ways.

First, the causative organism, in this case a virus, might not be able to complete its development in the body of the mosquito when the temperature is low for a considerable length of time.

Second, a low mean seasonal temperature might lower the general metabolism of the mosquito to such an extent that the intake of food during its lifetime would be restricted to one feeding of blood. If hereditary transmission of the disease is excluded, an epidemic under those circumstances is impossible.

Third, on comparatively cool days mosquitoes, once they have started feeding, persist until they have completed the blood meal. Under hot humid conditions, however, they are easily disturbed and will then feed partially on the same person two or more times or on two or more persons within a rather short time.

Fourth, cool temperature over a long period of time may bring about a shift in the time of emergence of possible vectors. Thus in 1941, *Aedes dorsalis*, *A. nigromaculis*, *A. vexans*, *Culex tarsalis*, and *Culiseta inornata* appeared in late July and early August, while in 1942 the last two failed to appear and the emergence of the first three took place near the middle of September. The season was then too late for an epidemic.

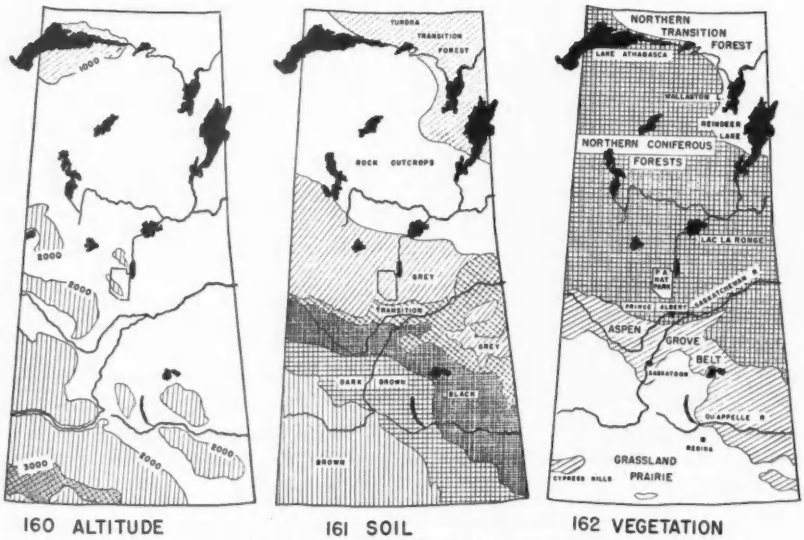
Fifth, although in cool wet seasons such as 1942, mosquitoes may be very abundant, their duration, if the rainfall comes in sudden heavy downpours, is short. It is then possible that the incubation period of the causative organism is longer than the life of the mosquito.

Sixth, in cool wet seasons the one generation aedine mosquitoes predominate, while in warm, comparatively dry seasons *Culex tarsalis*, *Culiseta inornata*, and the aedine mosquitoes with continuous development predominate. The latter group has been implicated in the transmission of the disease.

Seventh, in a hot season *Culex tarsalis* and *Culiseta inornata*, the species regarded as the two most important vectors of encephalomyelitis, complete their life cycle more quickly and thus may build up large populations by mid- or late summer.

Eighth, the presence of greater populations of *Culex tarsalis* in hot seasons is likely to increase the infectivity rate, since this species has been found to feed readily on birds which are regarded as hosts of the disease.

Although additional factors may be found to play a part in the transmission of Western equine encephalomyelitis, the evidence for the theory of mosquito transmission from the researches of Hammon and his co-workers is so convincing that the disease must be classed as mosquito borne. With Eastern equine encephalomyelitis and St. Louis encephalitis it belongs in a group of diseases known as the arthropod-borne virus encephalitides.



160 ALTITUDE 161 SOIL 162 VEGETATION

FIGS. 160-162. Maps of Saskatchewan. 160, altitude; 161, soil; 162, vegetation.

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